

FIG. 1A

Cell Sheet Constructs

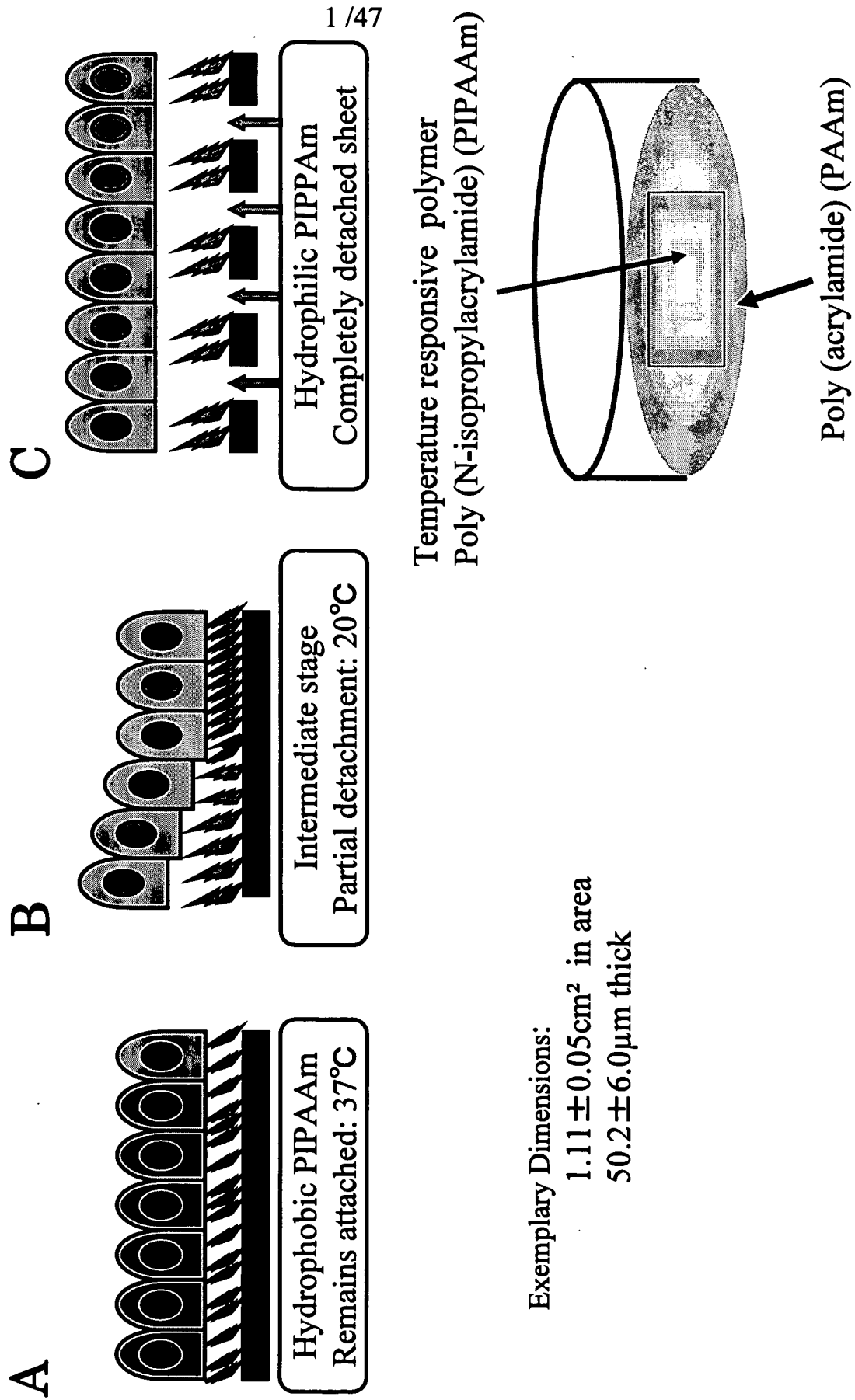
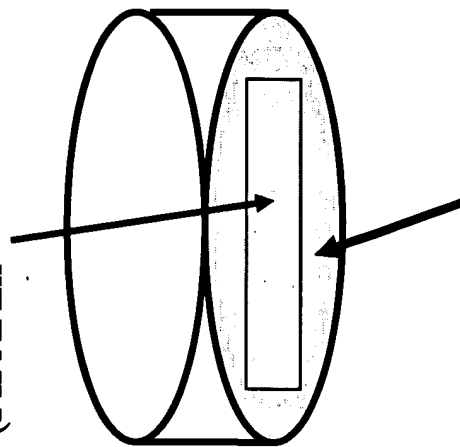


FIG.1B

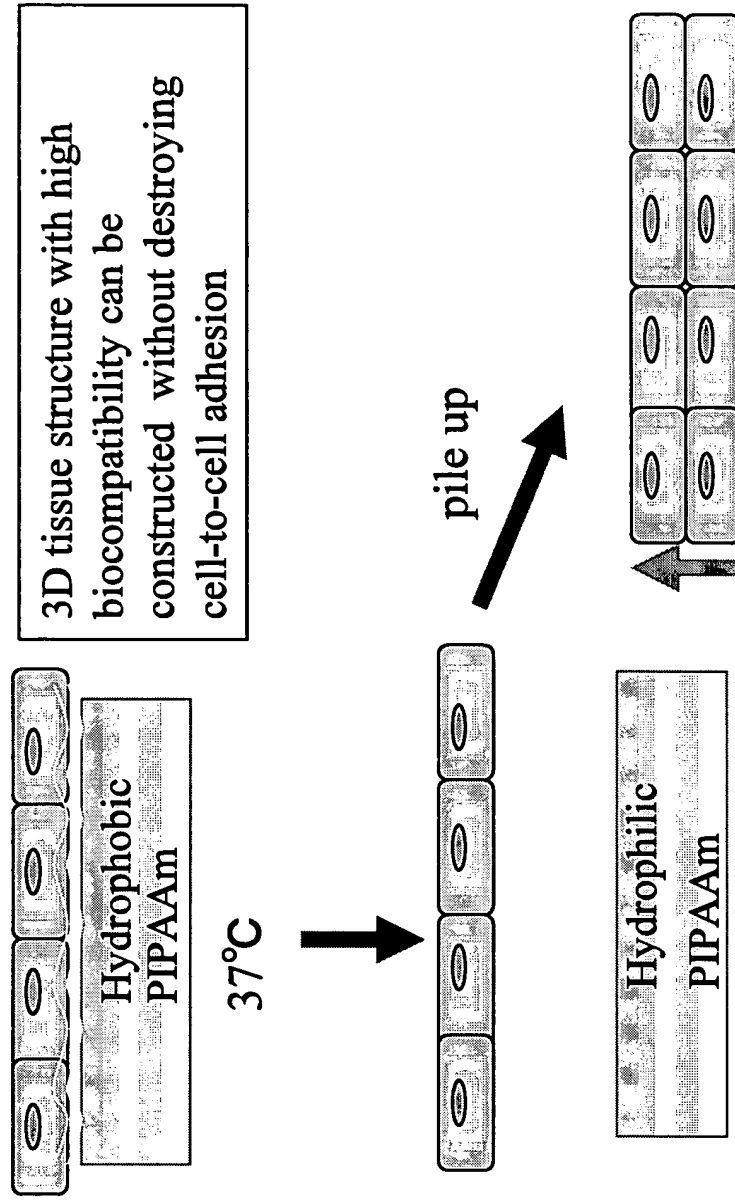
Poly (N-
isopropylacrylamide)
(PIPAAm)



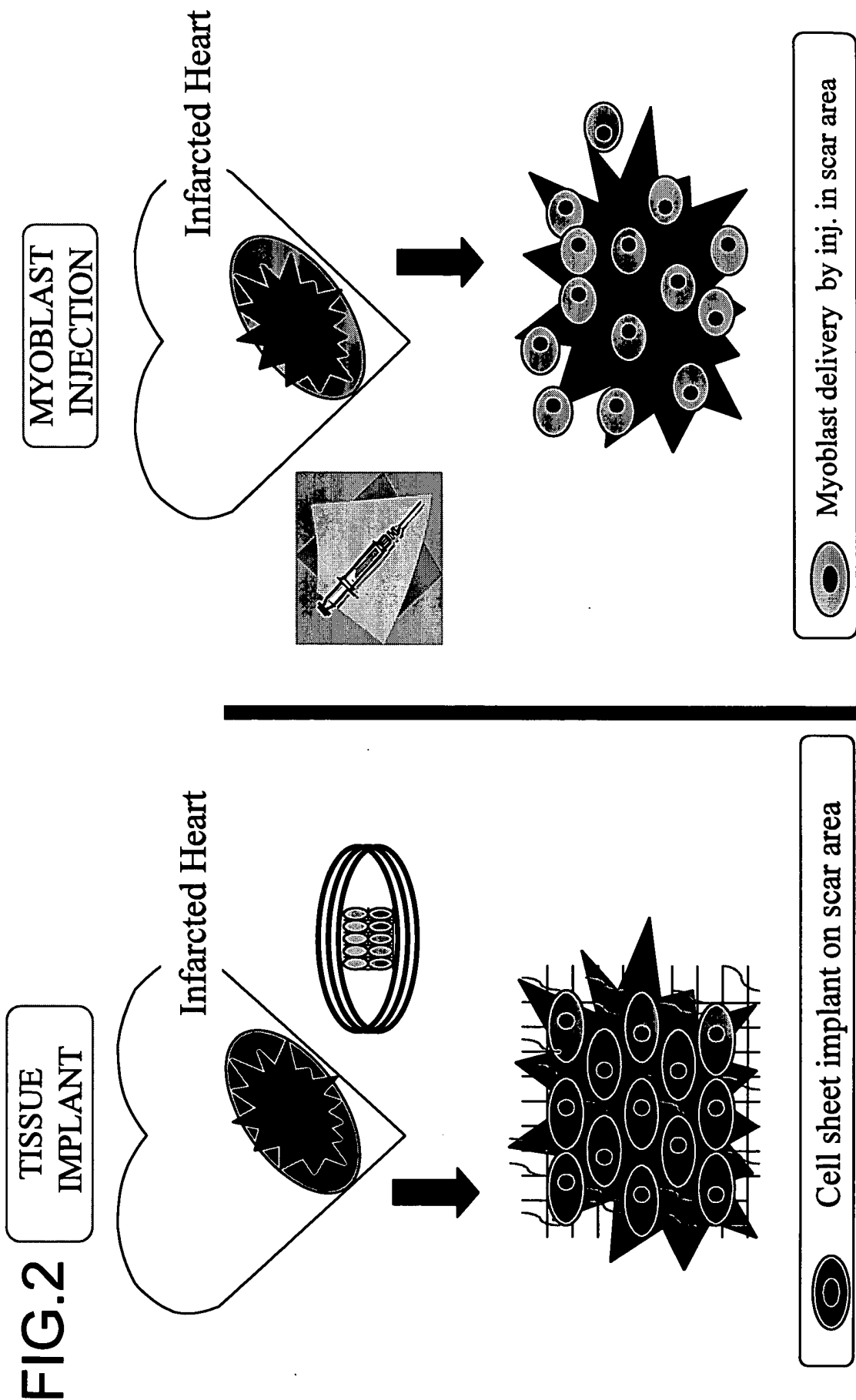
Polyacrylamide (PAAm)

No cell adhesion

Temperature responsive culture dish



32°C or less: detached as a sheet



Experimental Protocol

FIG.3

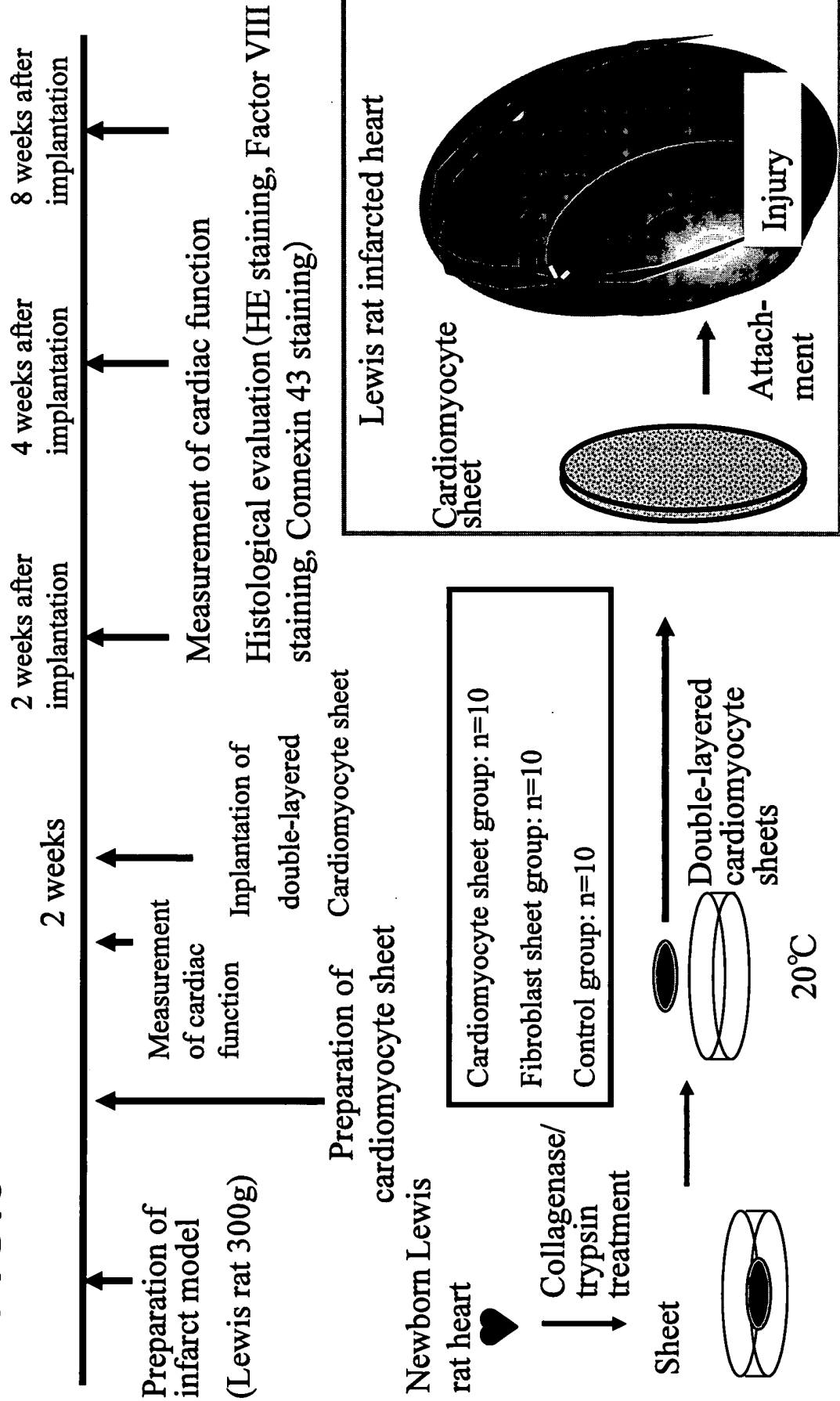


FIG.4 Regenerative therapy for cardiac
muscle by cell transplantation

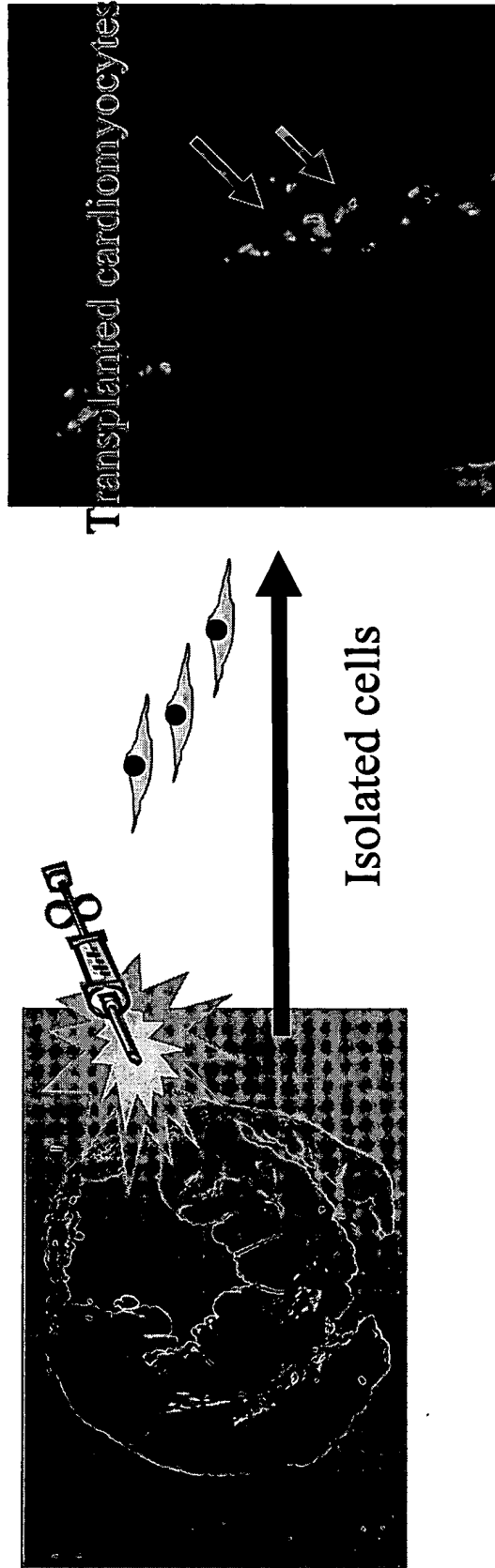


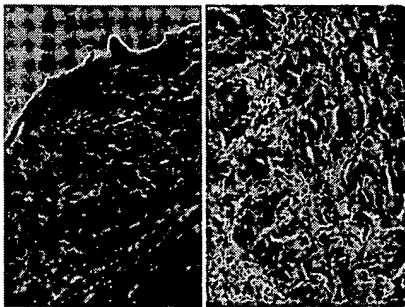
FIG.5 Problems with tissue transplantation

Cardiac muscle graft with scaffold

Alignment and cell-to-cell adhesion of transplanted cells within scaffold

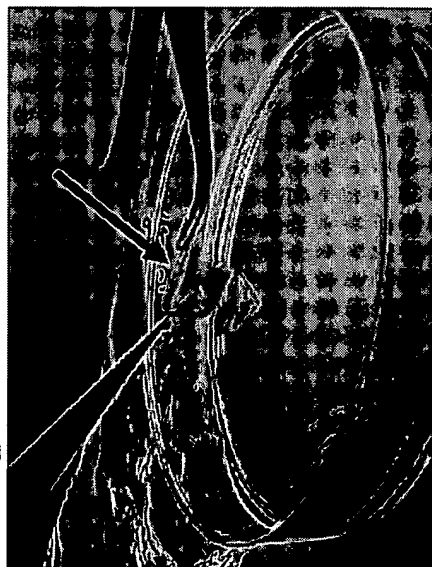
Changes in scaffold in organism : elicitation of inflammation

Acceptance of scaffold by recipient's heart



Development of high biocompatible cardiac muscle graft without scaffold

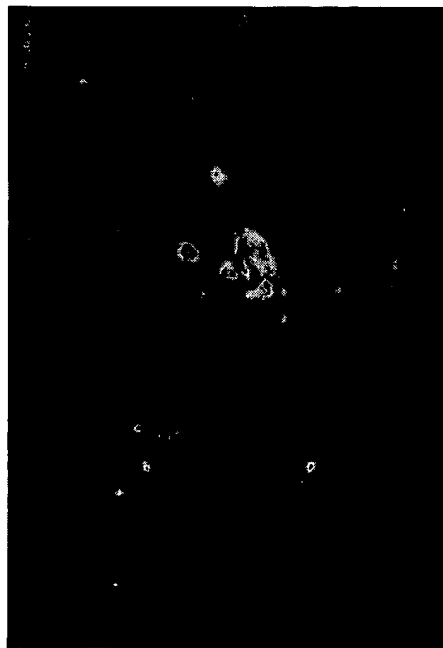
FIG.6 Implantation of cardiomyocyte sheet into infarcted heart



Cardiomyocyte sheet

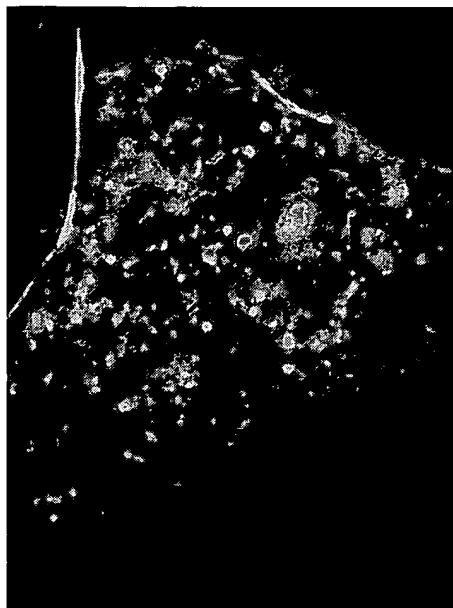


Implantation into rat infarct model



In vitro

Implantation of GFP rat newborn cardiomyocyte sheet



In vivo

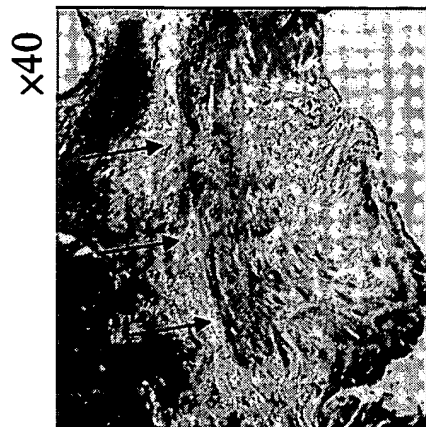
Implantation of GFP rat newborn cardiomyocyte sheet

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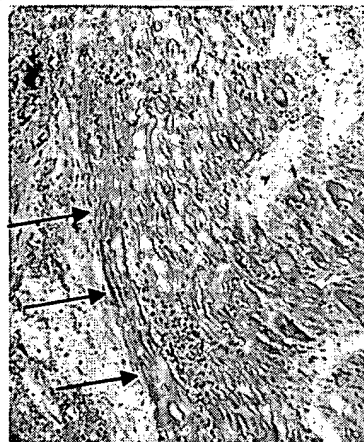
Tissue

FIG. 7

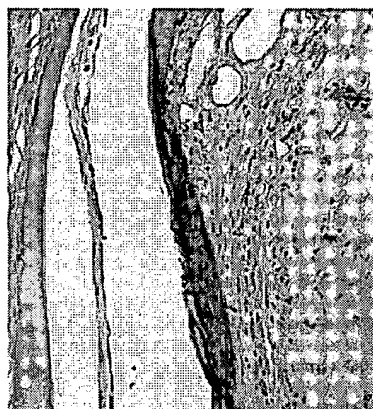
8 weeks after
implantation



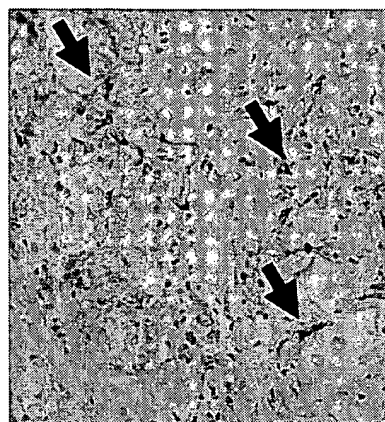
x 100



x 200



HE staining

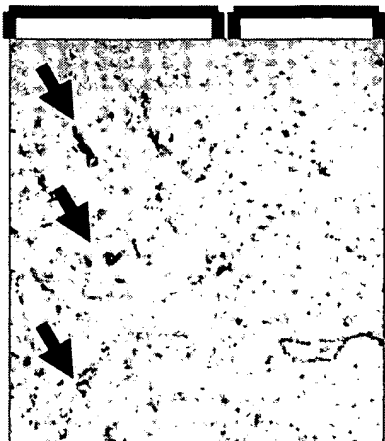


Connexin 43



Cardiomyocyte sheet

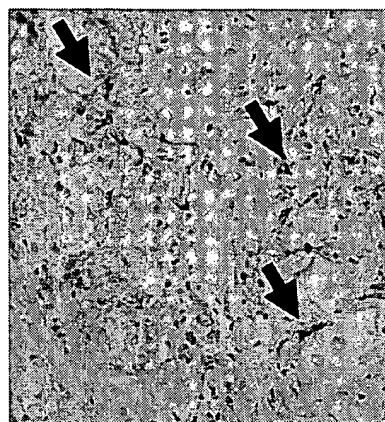
HE staining



Cardiomyocyte sheet

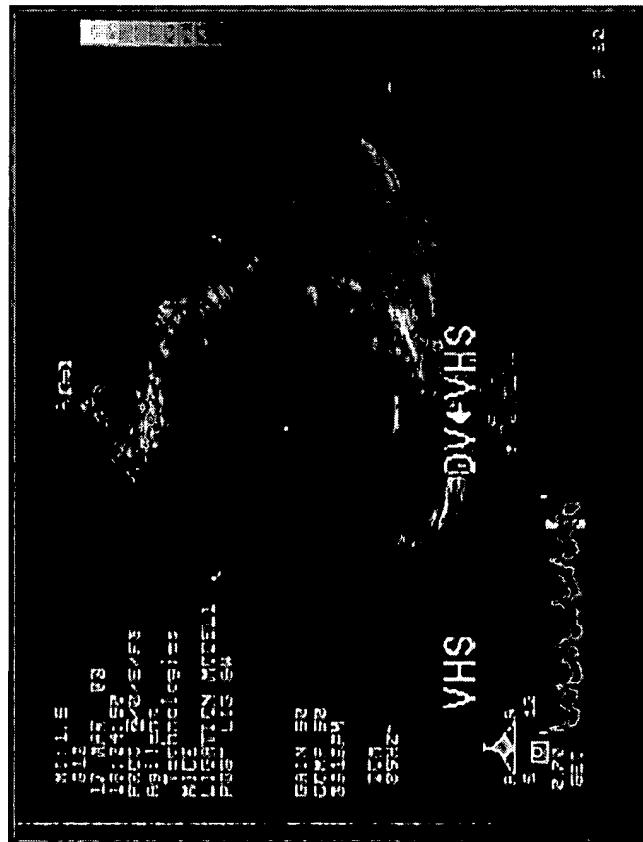
Recipient heart

Factor VIII staining

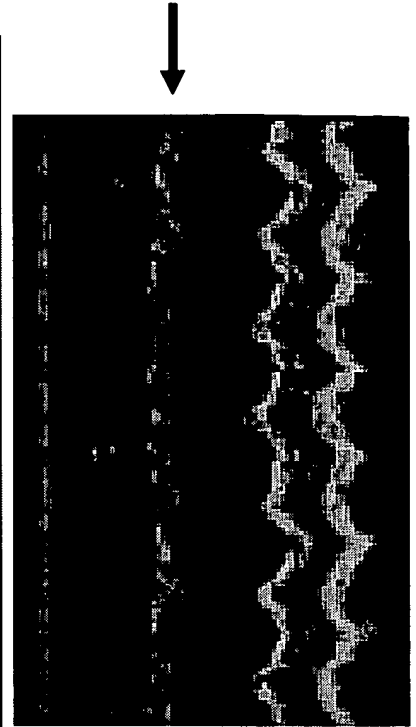
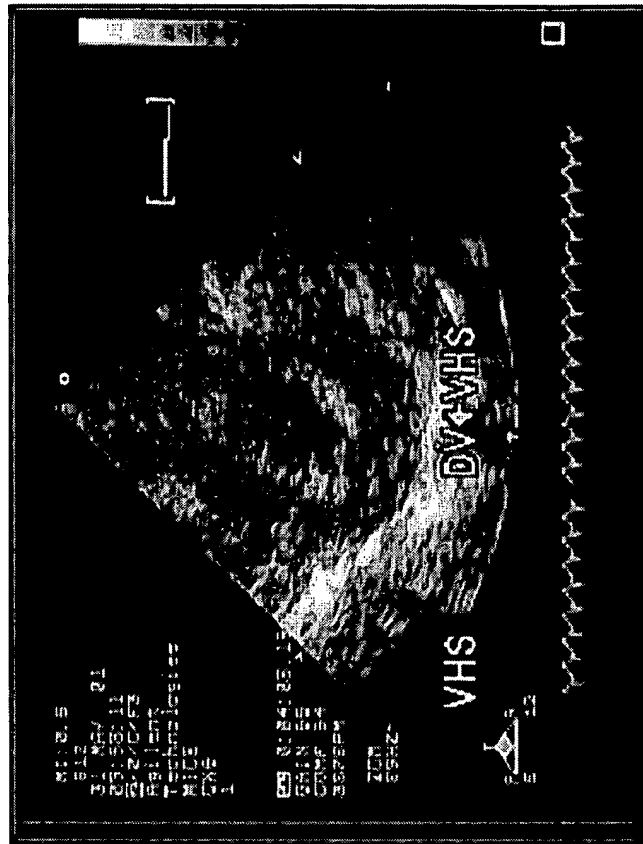


EIG 8

Control



Implantation of prosthetic tissue

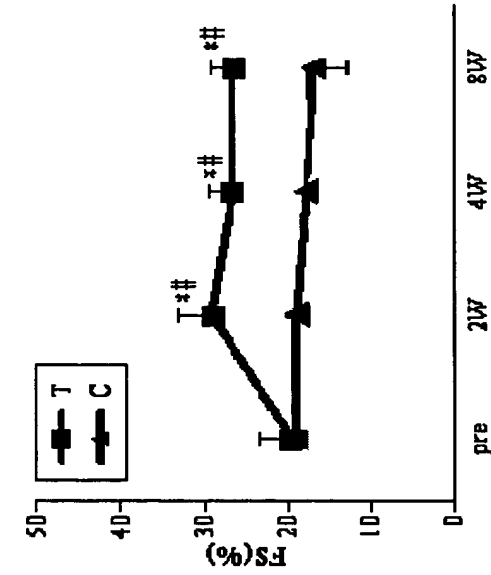


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FIG.9

Evaluation of cardiac function - 2

Fractional Shortening

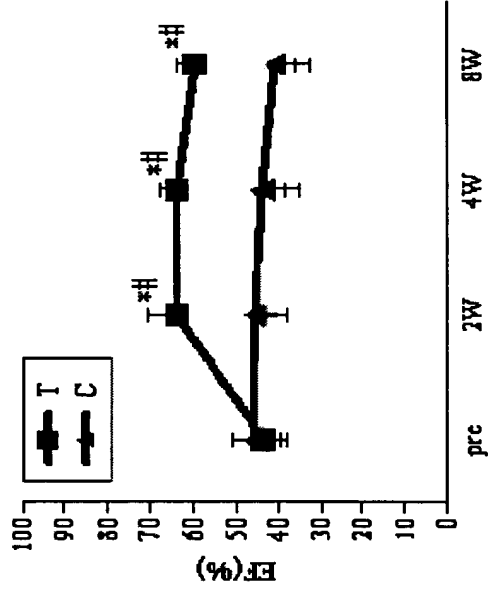


Cardiomycocyte sheet

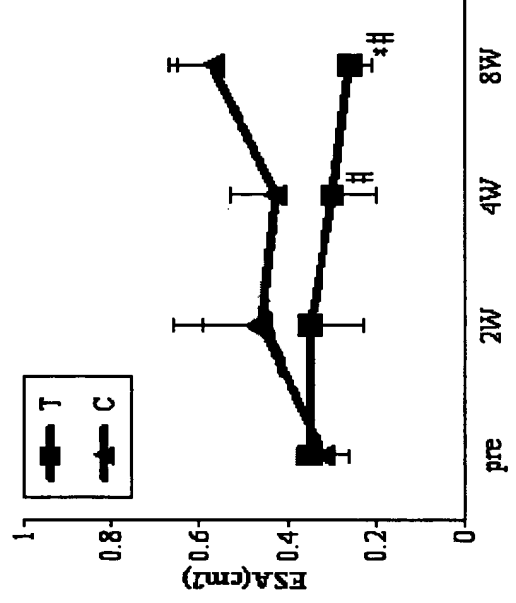
Control

*: p<0.05 to control

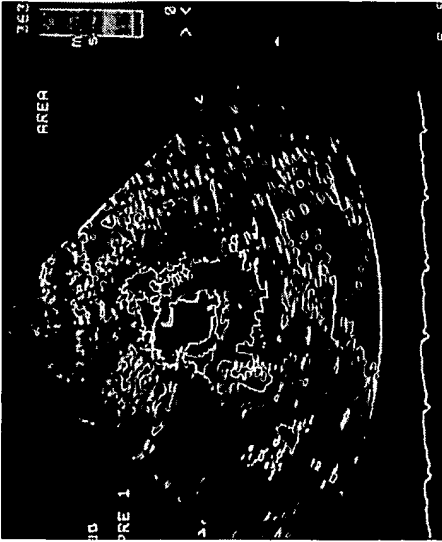
Ejection Fraction



End-systolic area



Base line



Implanted cardiomycocyte sheet

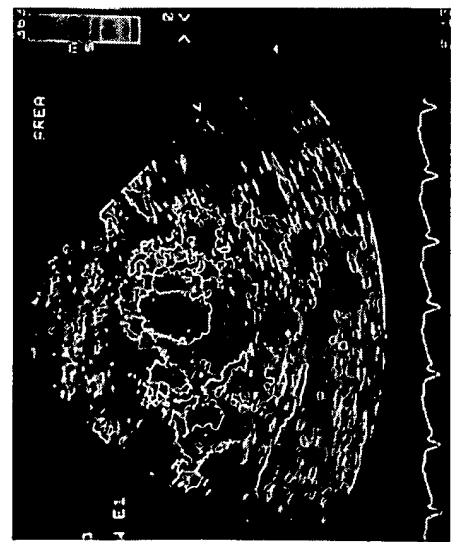
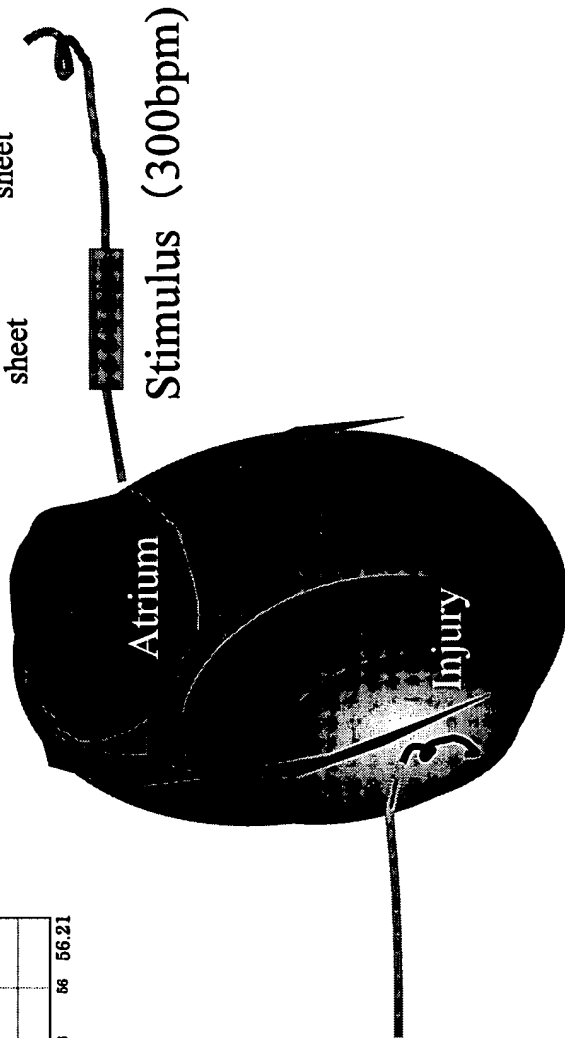
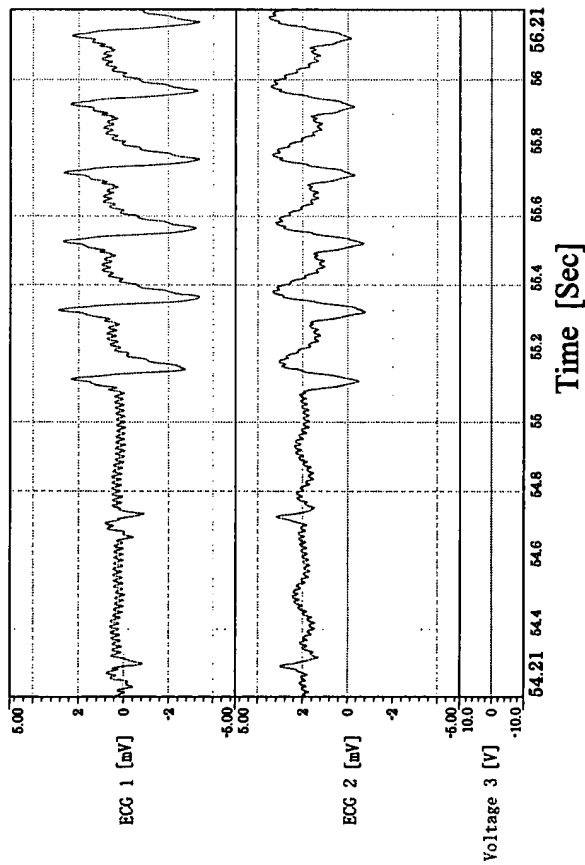
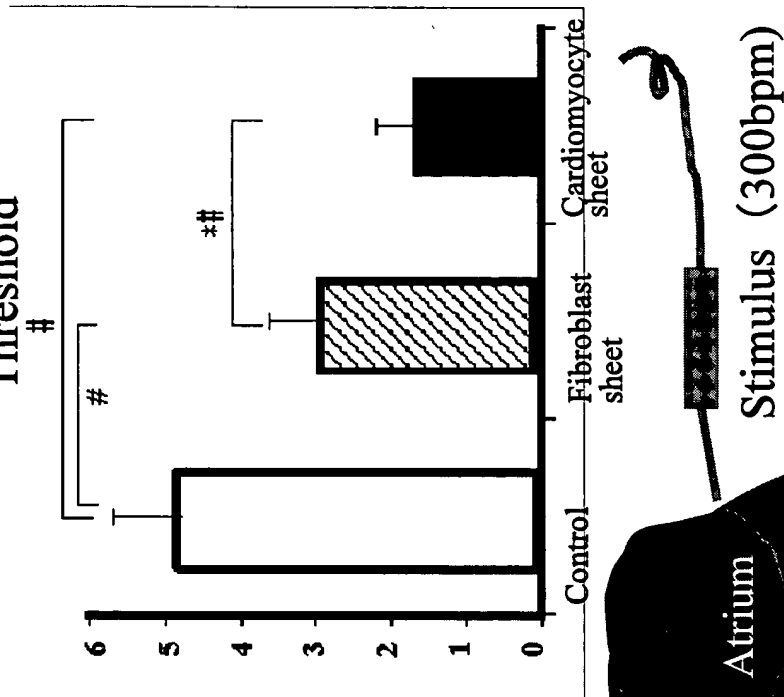


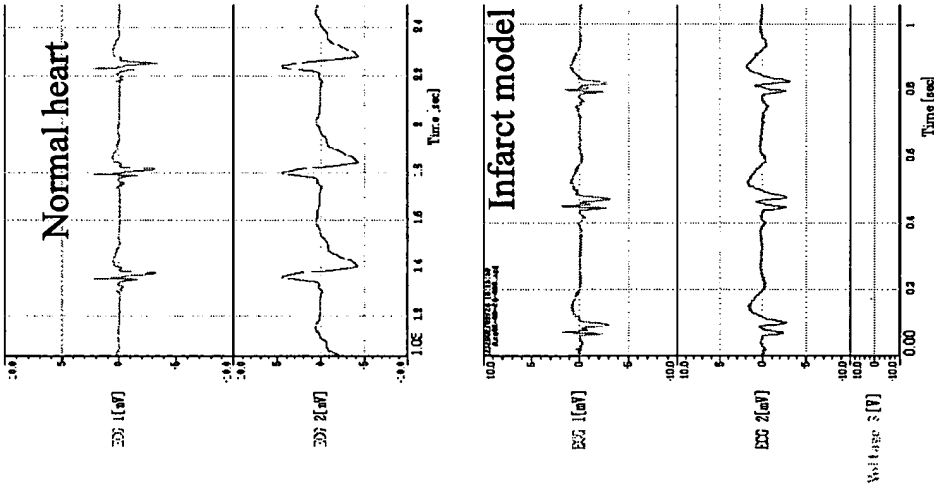
FIG.10

Electrophysiological Evaluation



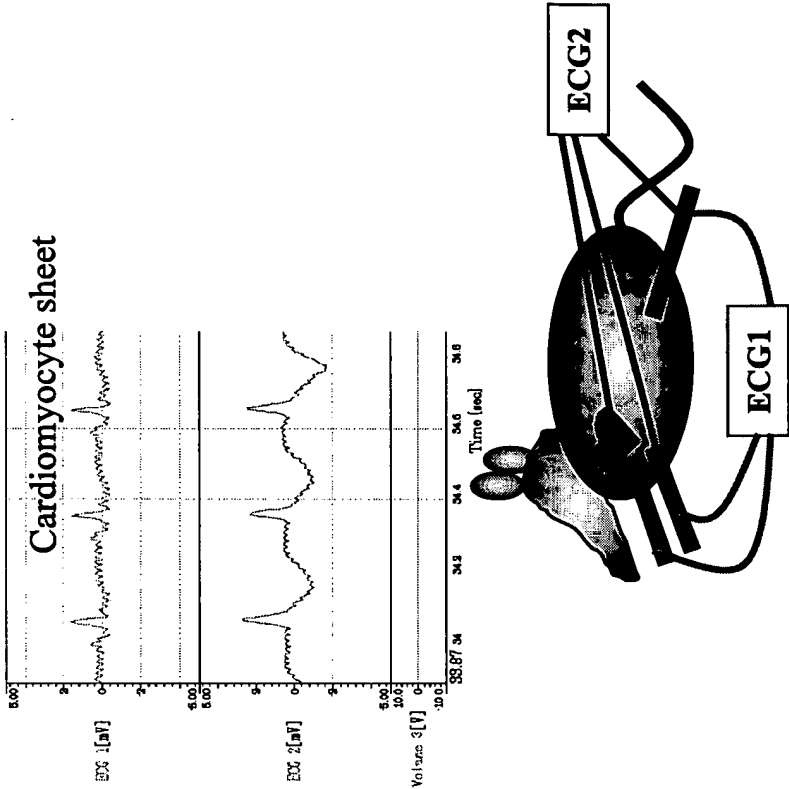
Buried electrode at
sheet implanted site

FIG.11



ECG 1:ECG (Surface)
ECG 2: Normal heart (anterior wall)
Ligation model (injured)
Prosthetic tissue implanted
(prosthetic tissue injured)

Electrophysiological Evaluation



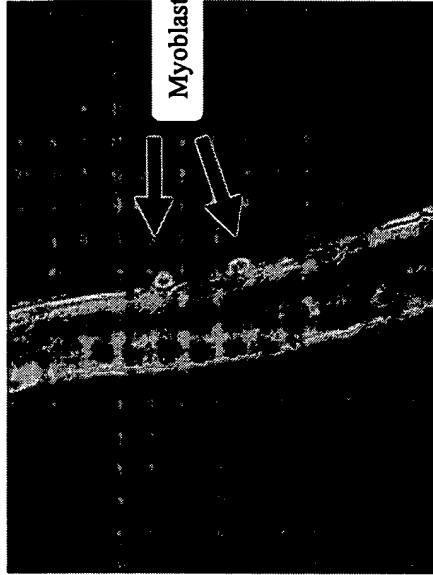
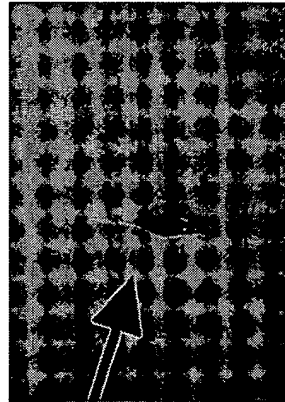
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FIG.12

Isolation and culture of myoblast



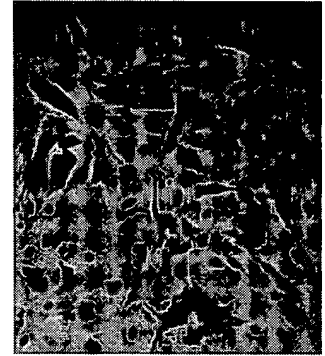
Harvested Muscle



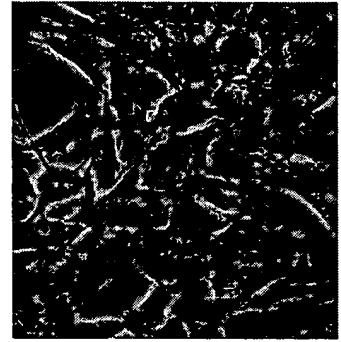
Collagenase 37°C, 30min



Desmin stained Myoblasts

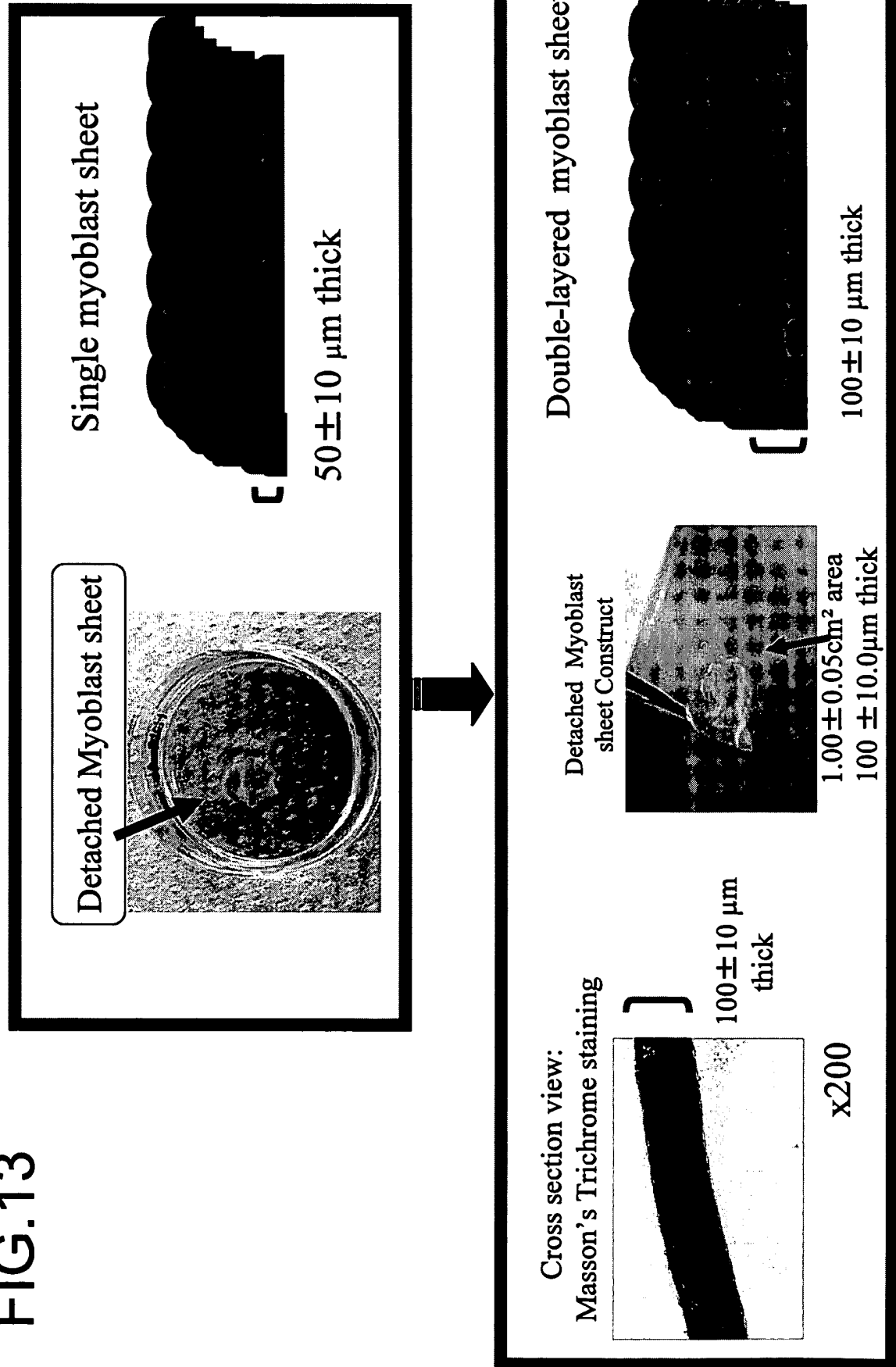


Myoblasts in culture



Methods: Myoblast Sheet Construction

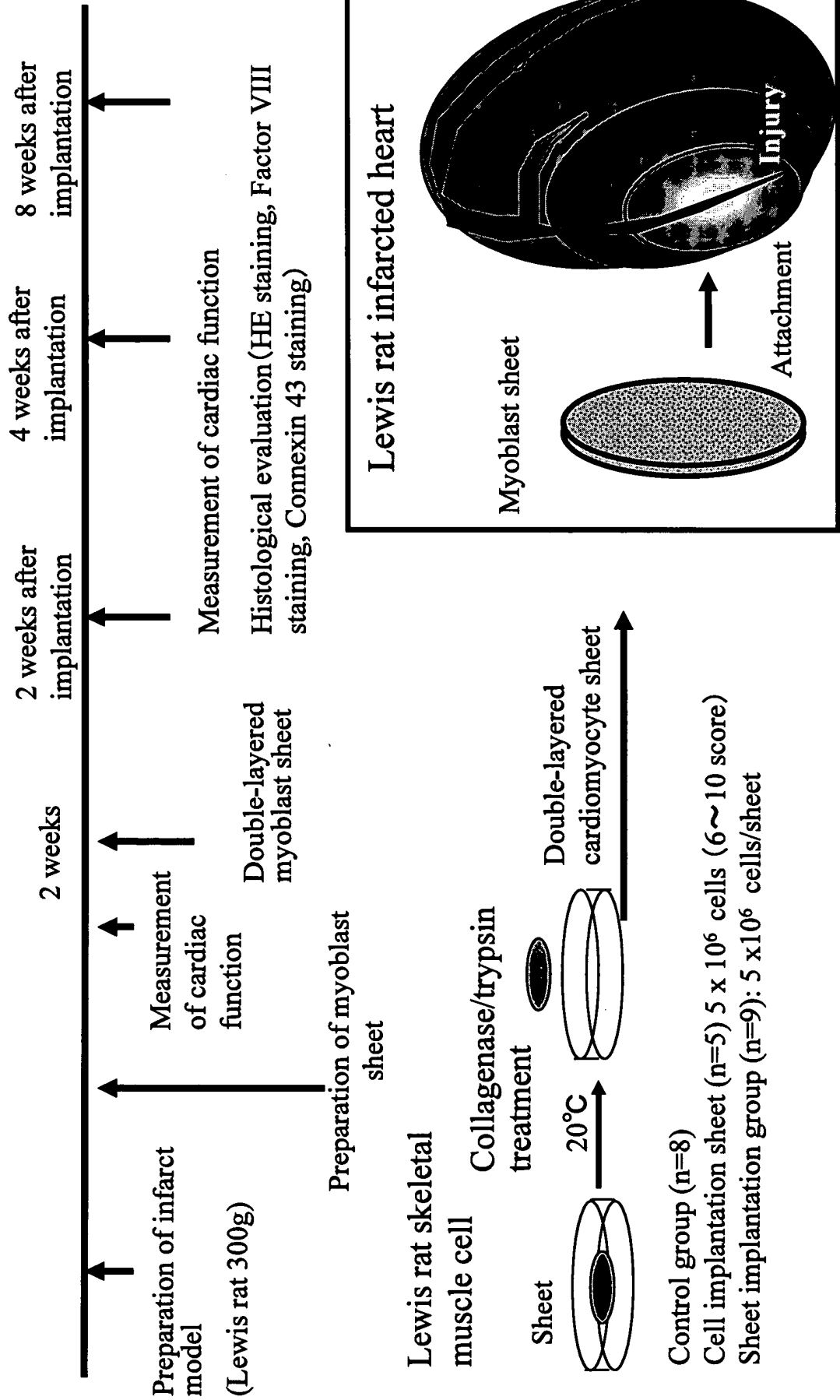
FIG.13



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FIG.14

Experimental Protocol



Myoblast sheet: 4W post implantation

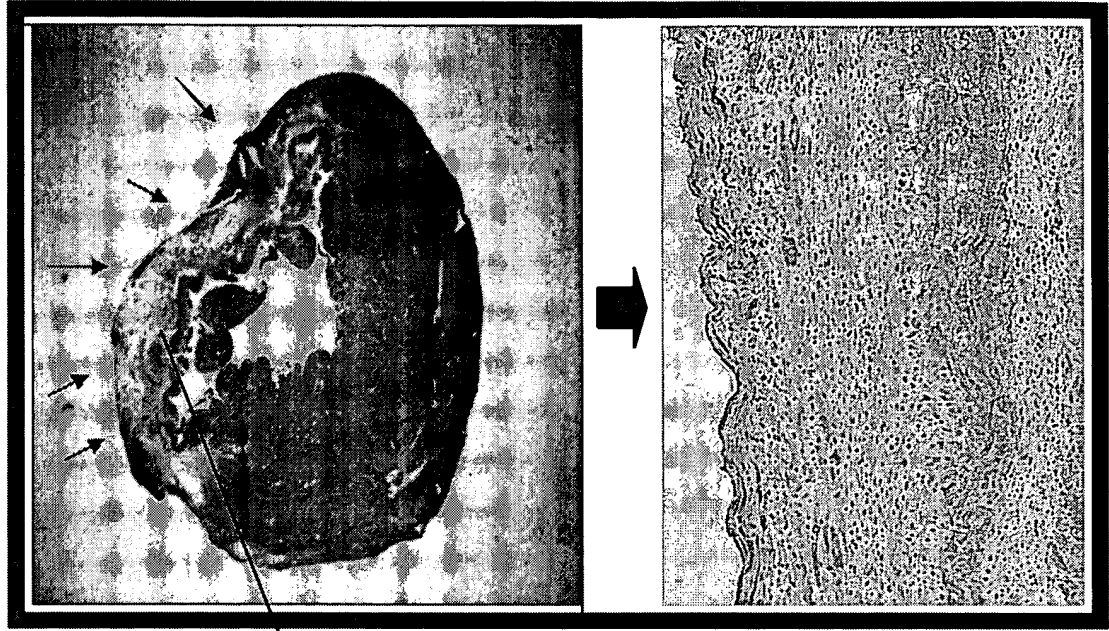
FIG.15

Implanted myoblasts



x1000

HE staining



x10

x200

FIG.16 Myoblast sheet Implantation procedure

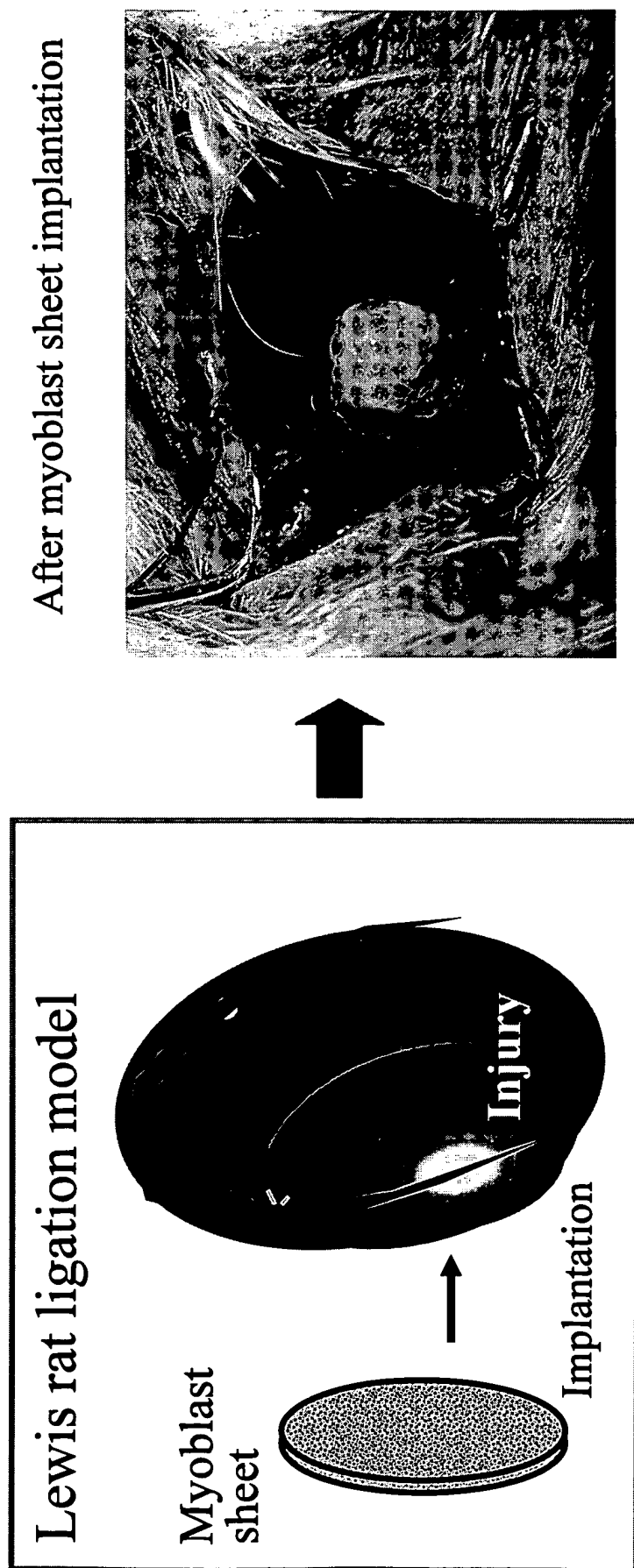


FIG.17 HistologyMasson's Trichrome staining

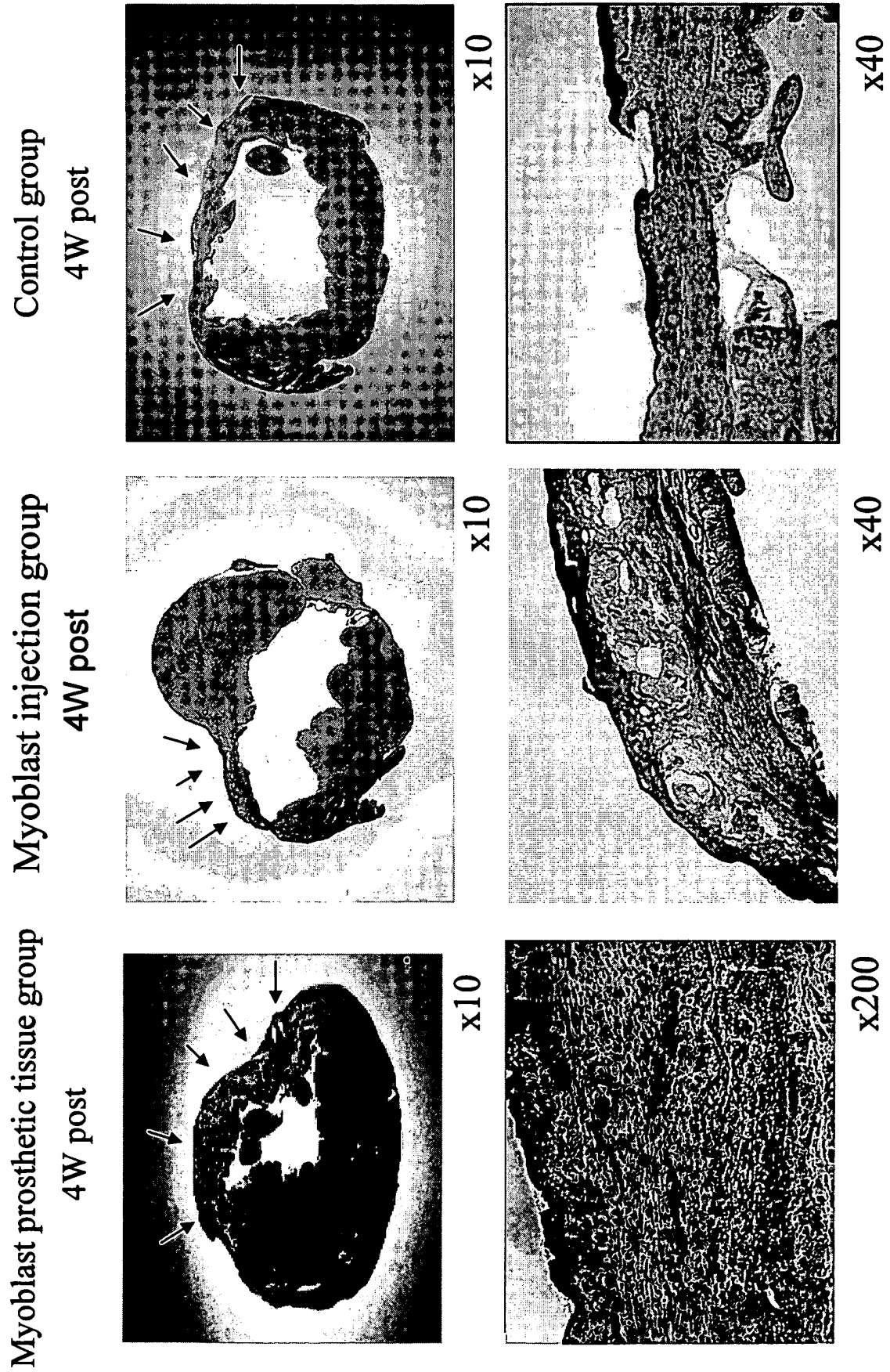
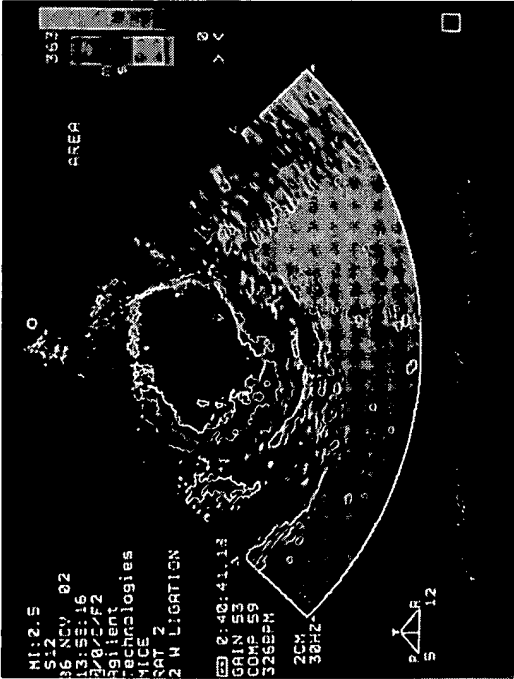
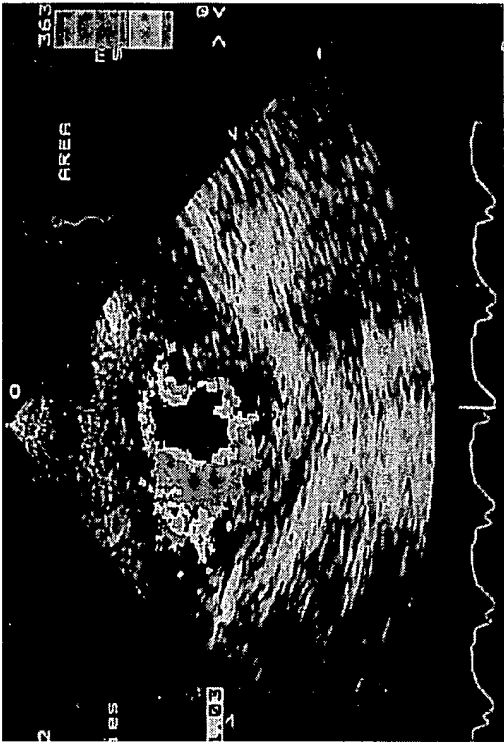


FIG.18 CKA



M-mode analysis

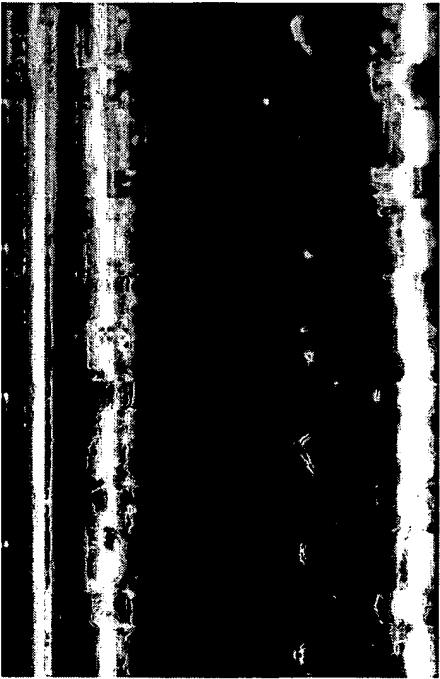
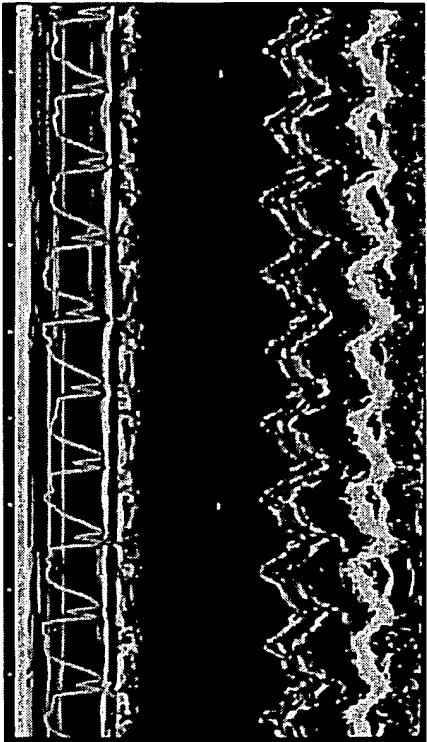
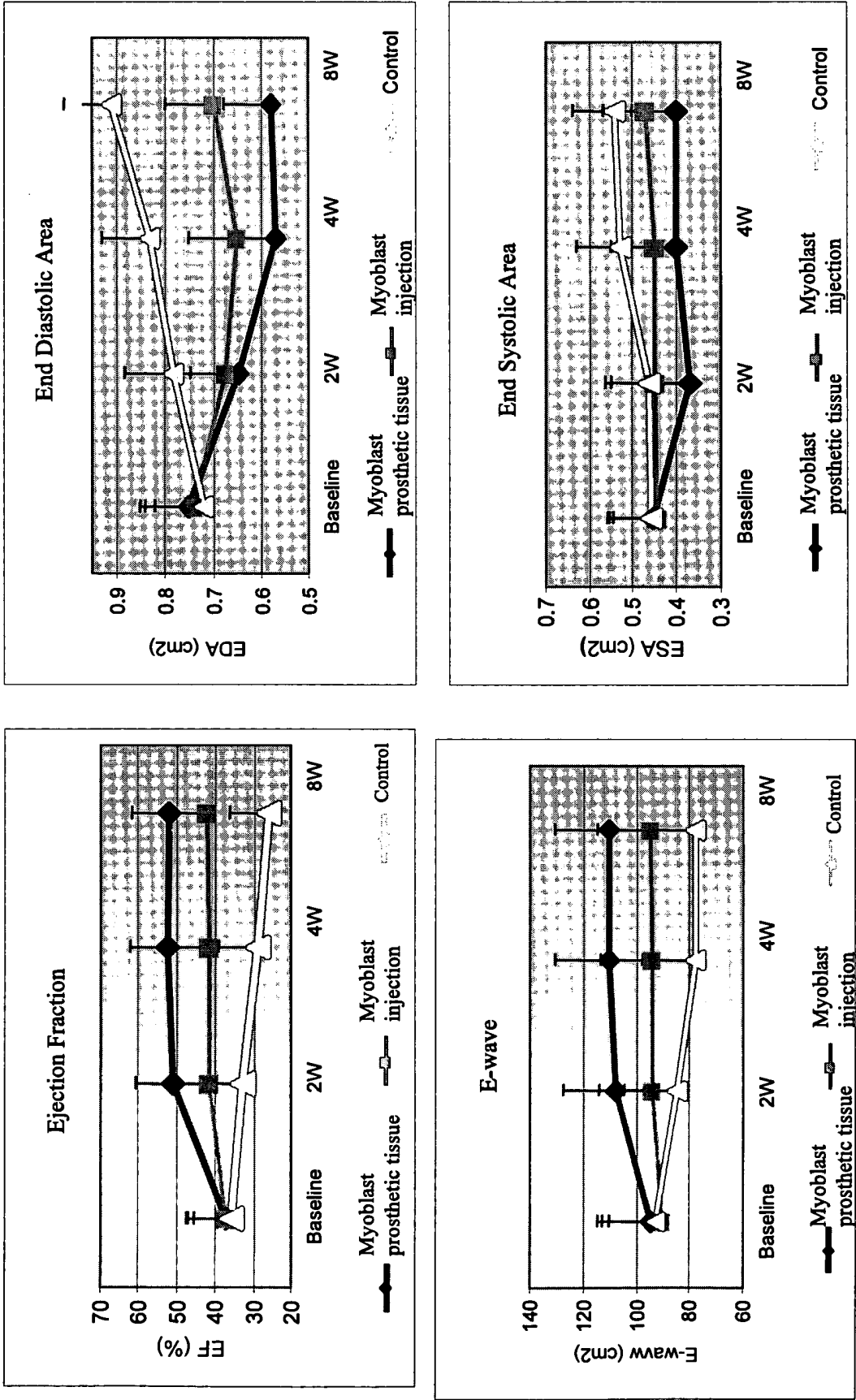


FIG.19



#P< 0.05 for control; *P< 0.05 to for injection needle group

Anterior Wall Thickness Comparison

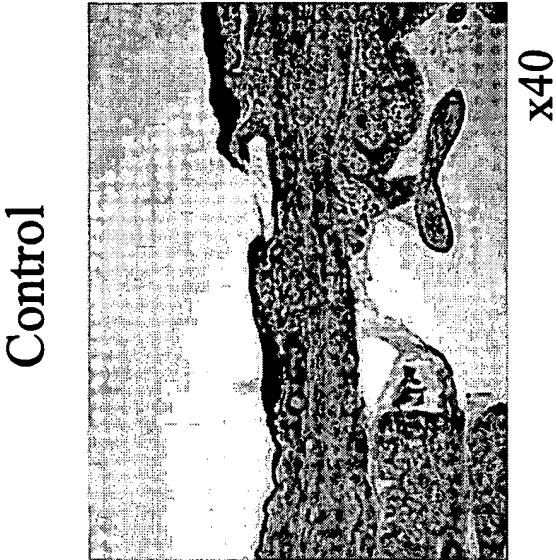
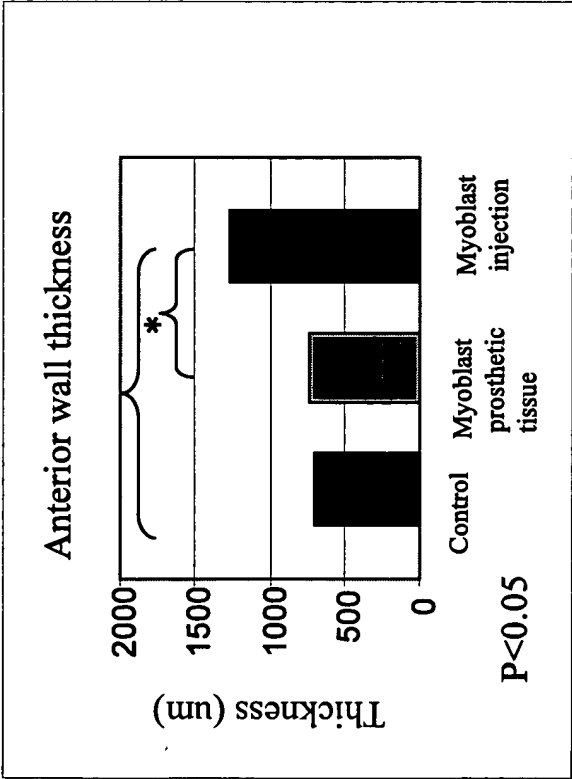
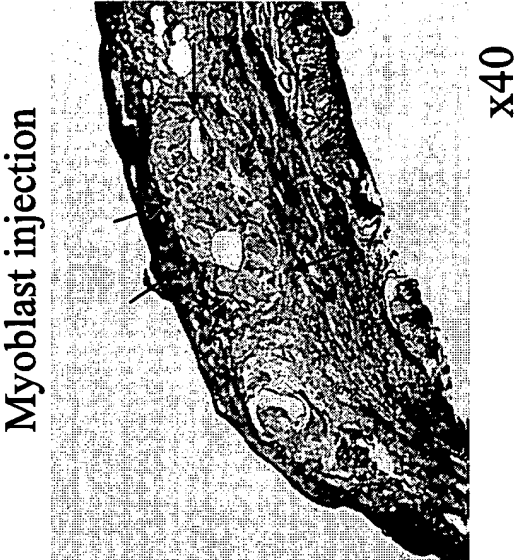
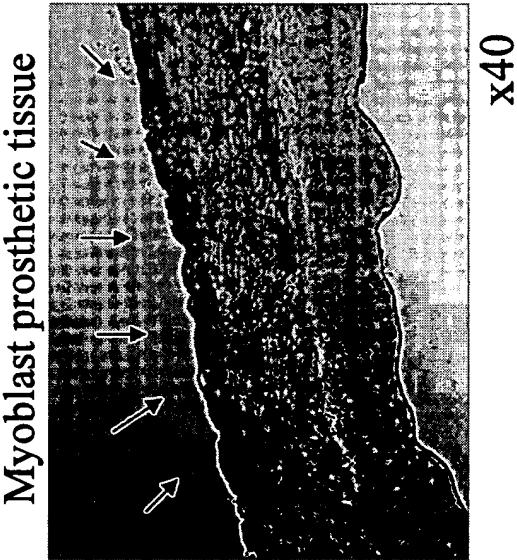


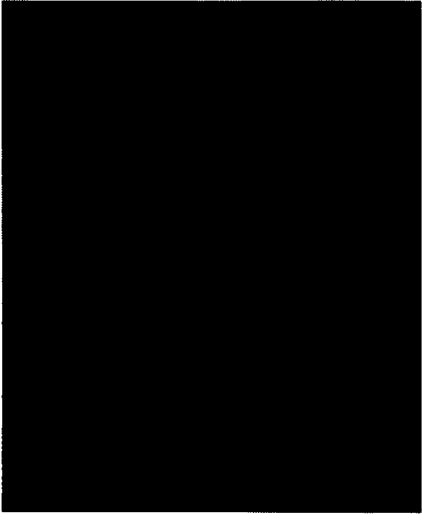
FIG.21

Myoblast sheet:
Desmin Staining



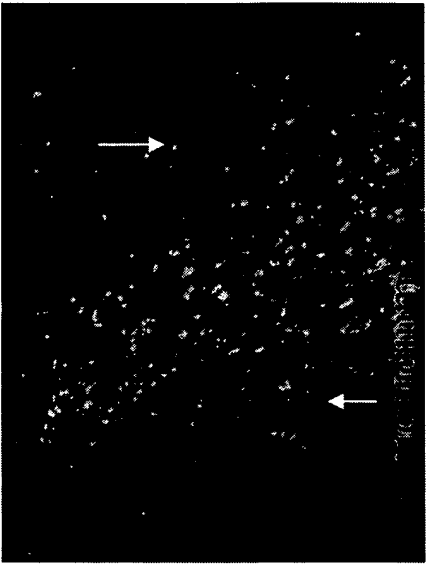
x100

Control group (GFP)



x100

Myoblast prosthetic
tissue group (GFP)



x100

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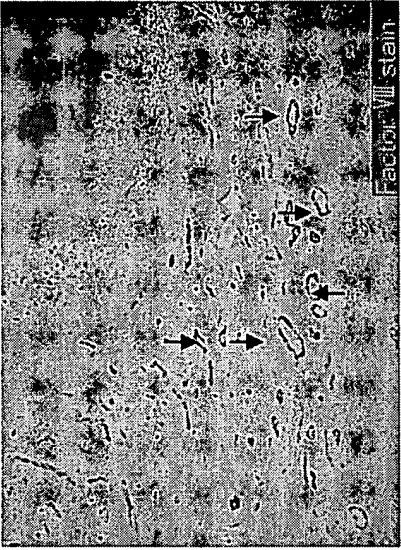
Factor VIII staining

Myoblast injection



x40

Myoblast prosthetic tissue



x40

Control



x40

FIG.22A



FIG.22B

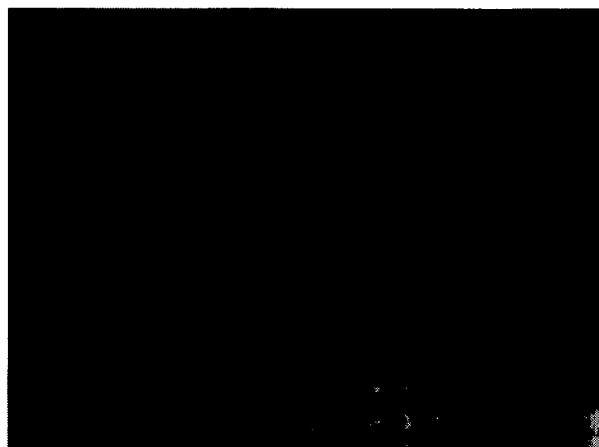


FIG.22C



FIG.22D

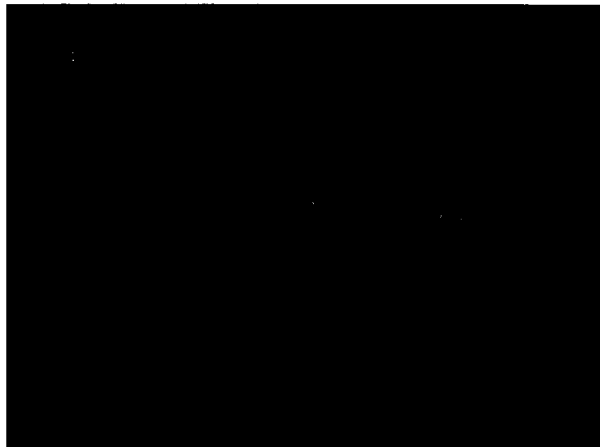


FIG.22E



FIG.22F



FIG.23A

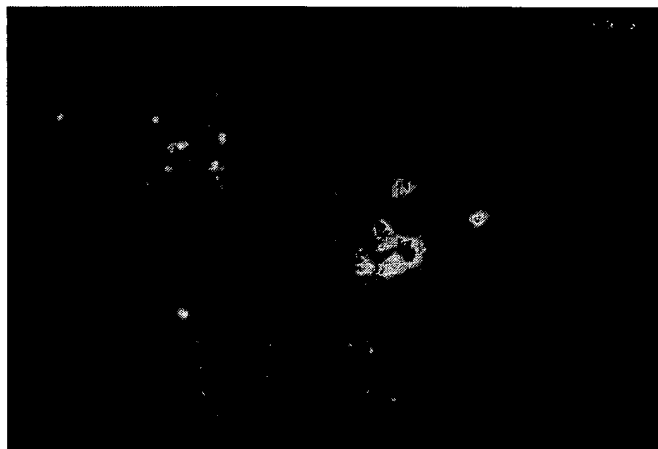


FIG.23B

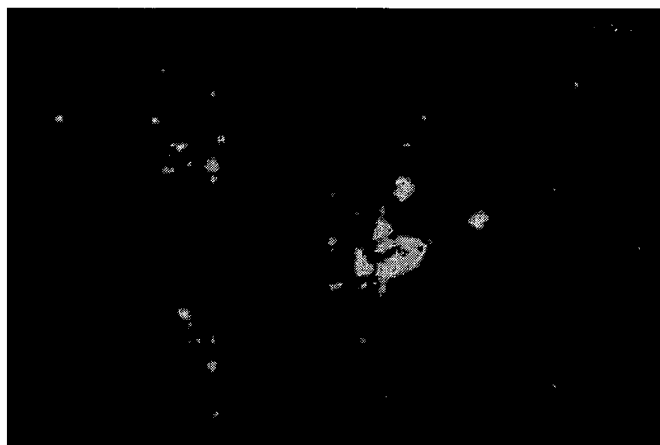


FIG.23C



FIG.24A

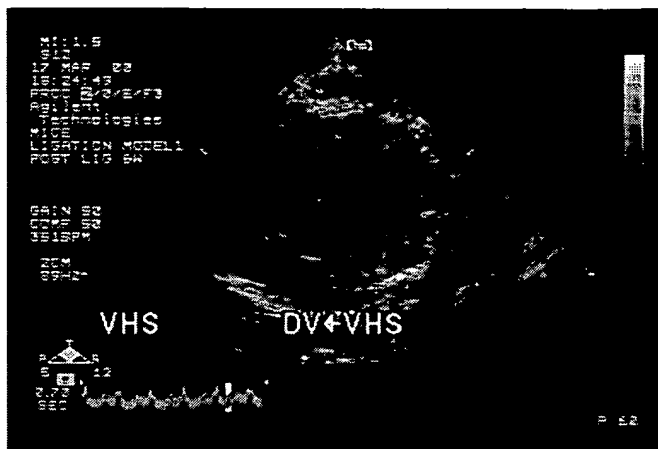


FIG.24B

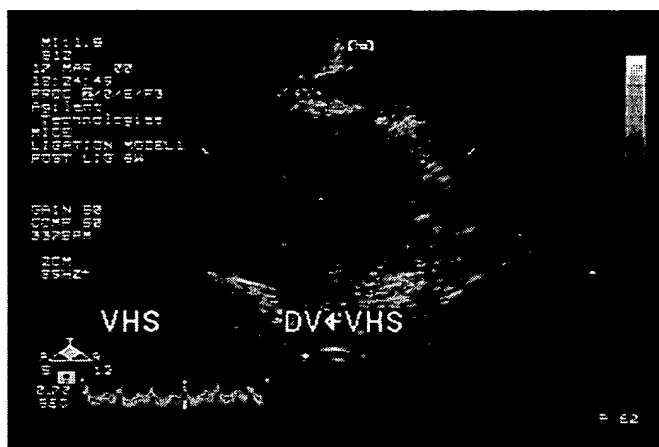


FIG.24C

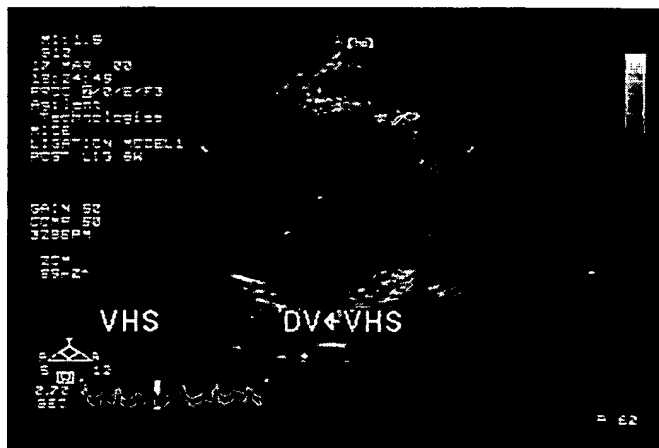


FIG.25A

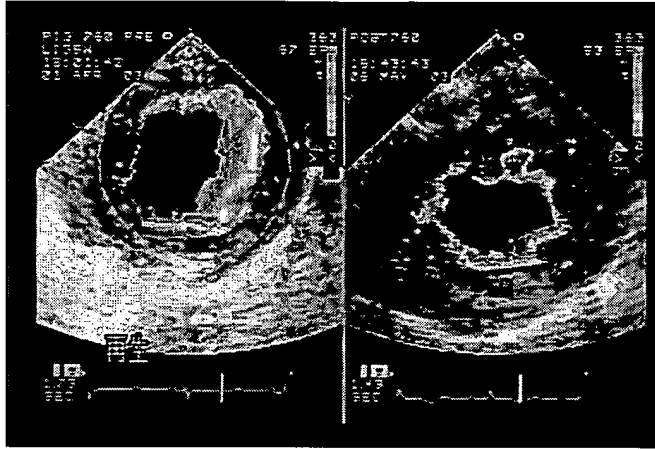


FIG.25B

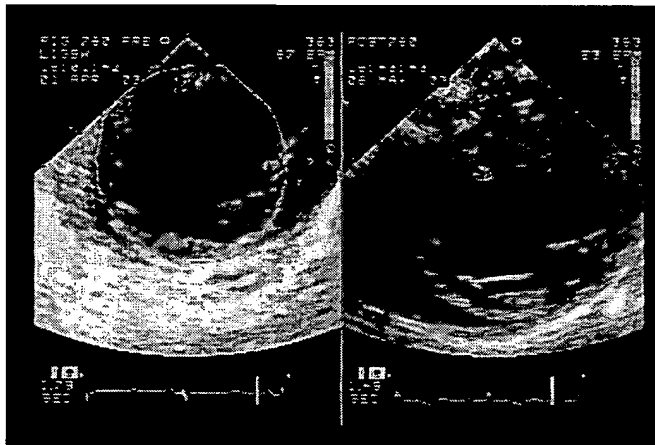


FIG.25C

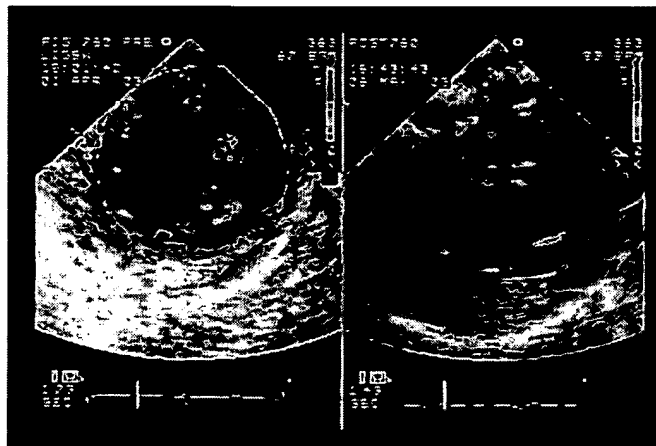


FIG.26A

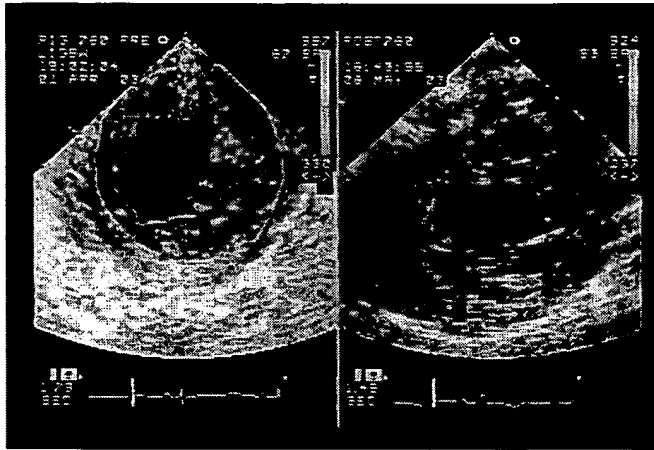


FIG.26B

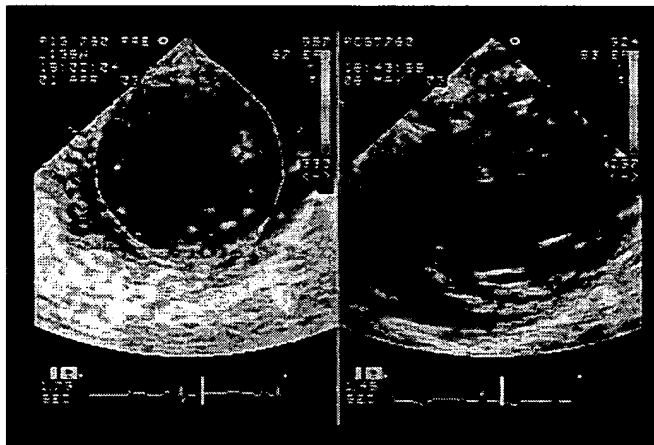


FIG.26C

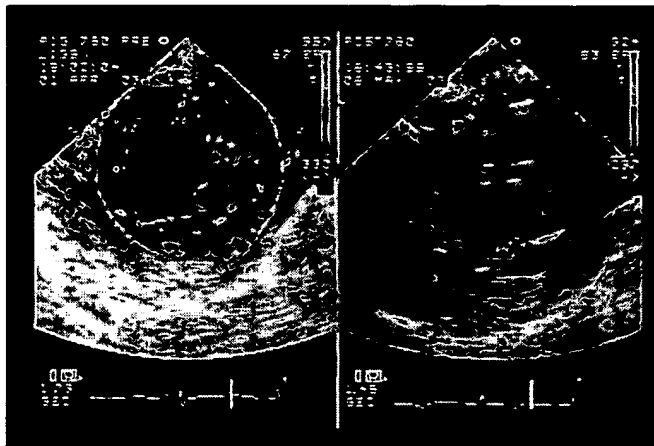


FIG.27A

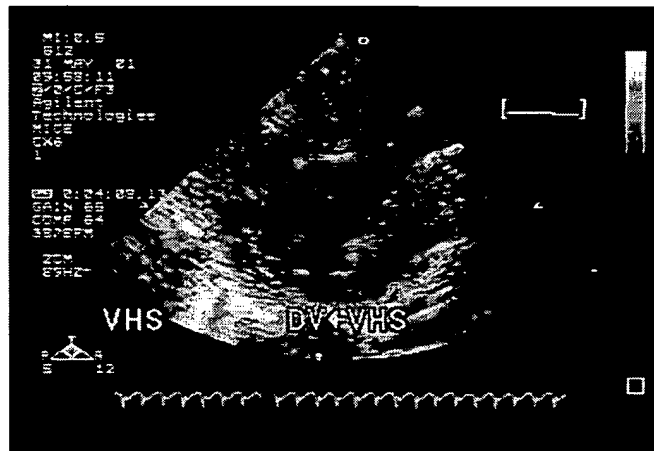


FIG.27B

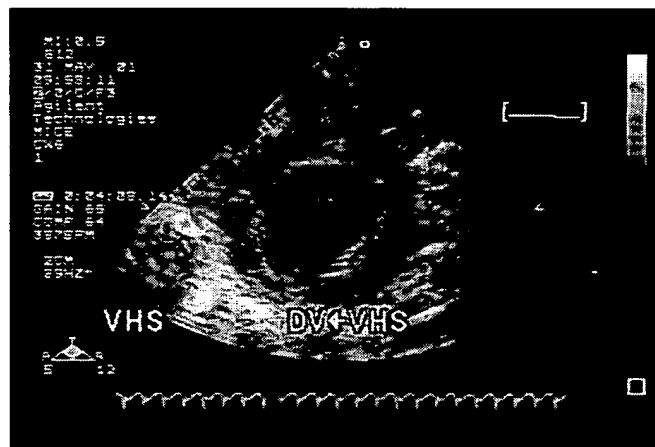


FIG.27C

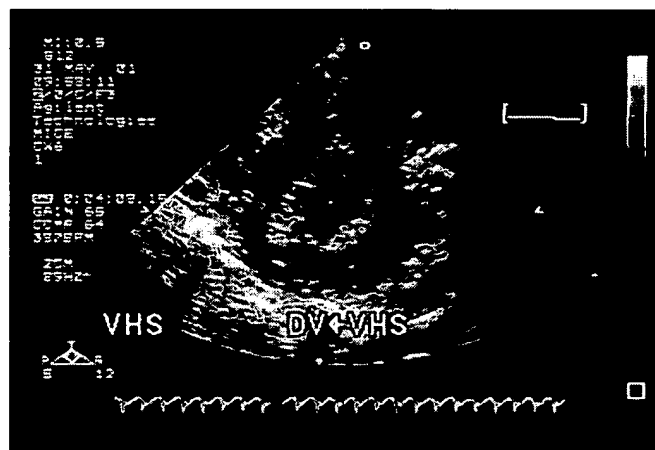


FIG.28

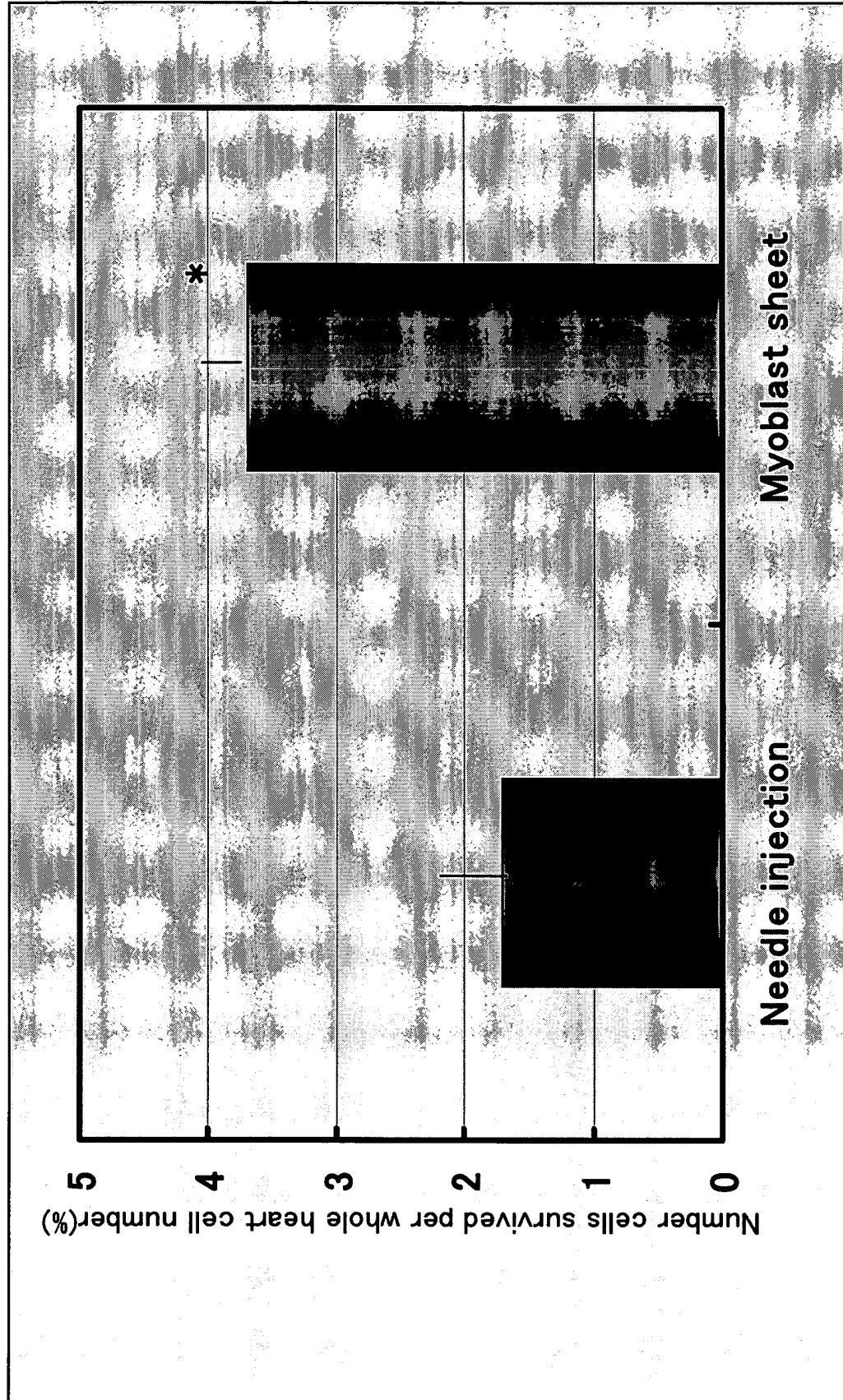
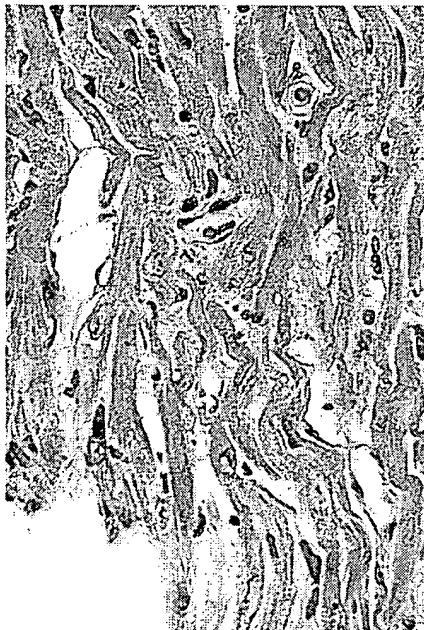


FIG.29

Masson's Trichrome staining x400



HE staining x400



MHC fast x400



MHC slow x400

FIG.30A Tissue (Masson's Trichrome staining)

Myoblast sheet
4W post implantation

x10



Myoblast implantation
(needle injection)

4W post implantation x10



Control

x10



x40

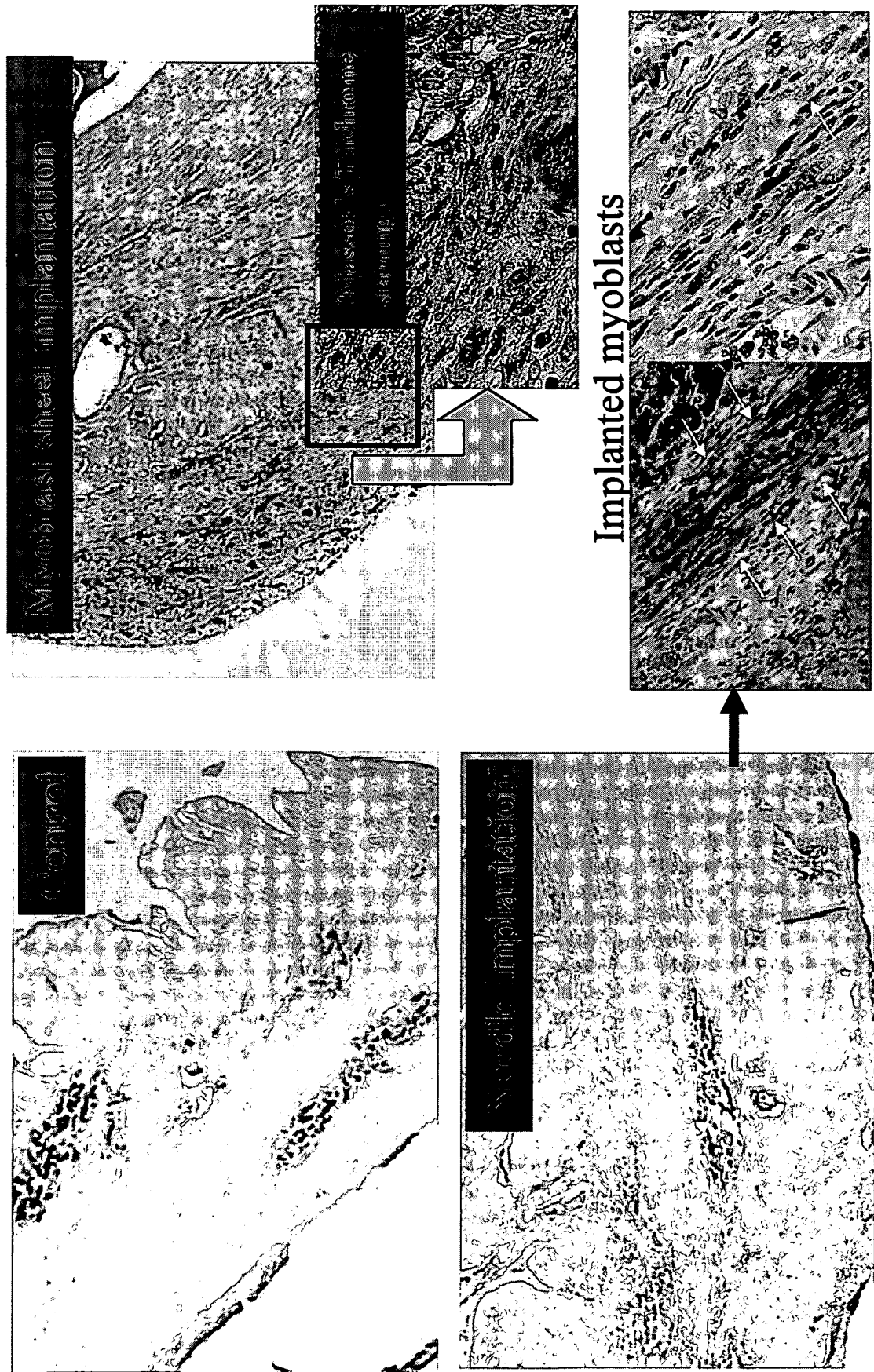


x40



x40

FIG.30B



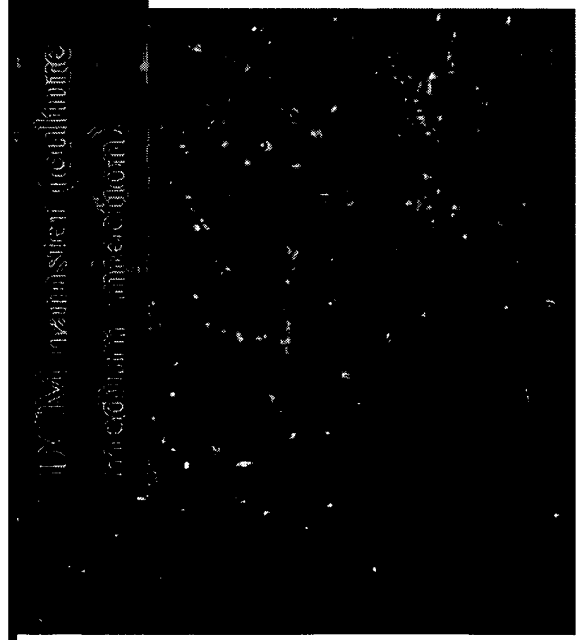
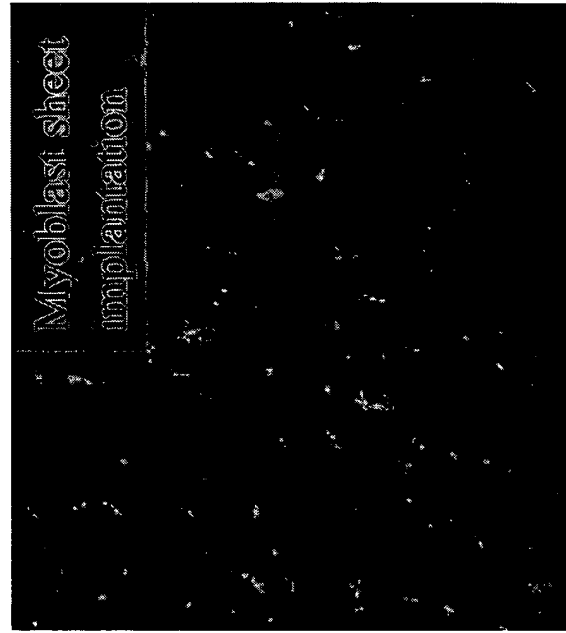


FIG.30C

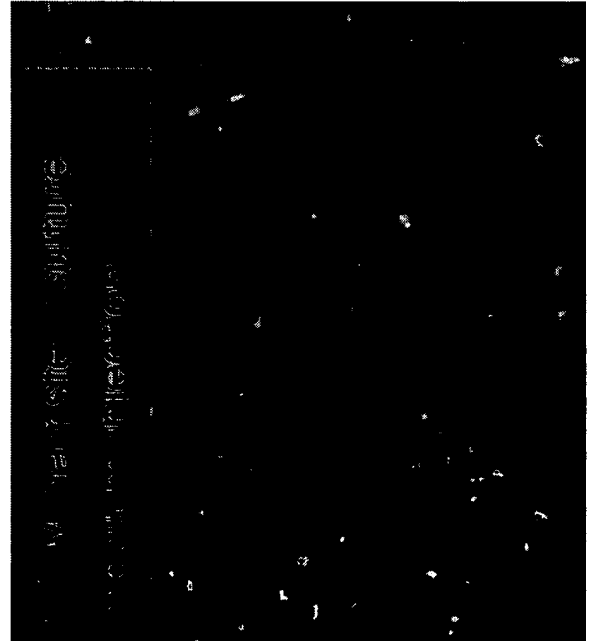
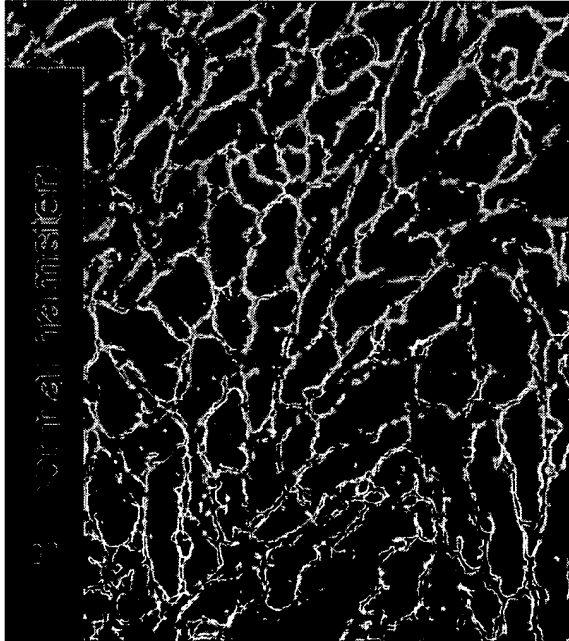


FIG.30D

FIG.31 Survival rate of implanted cell

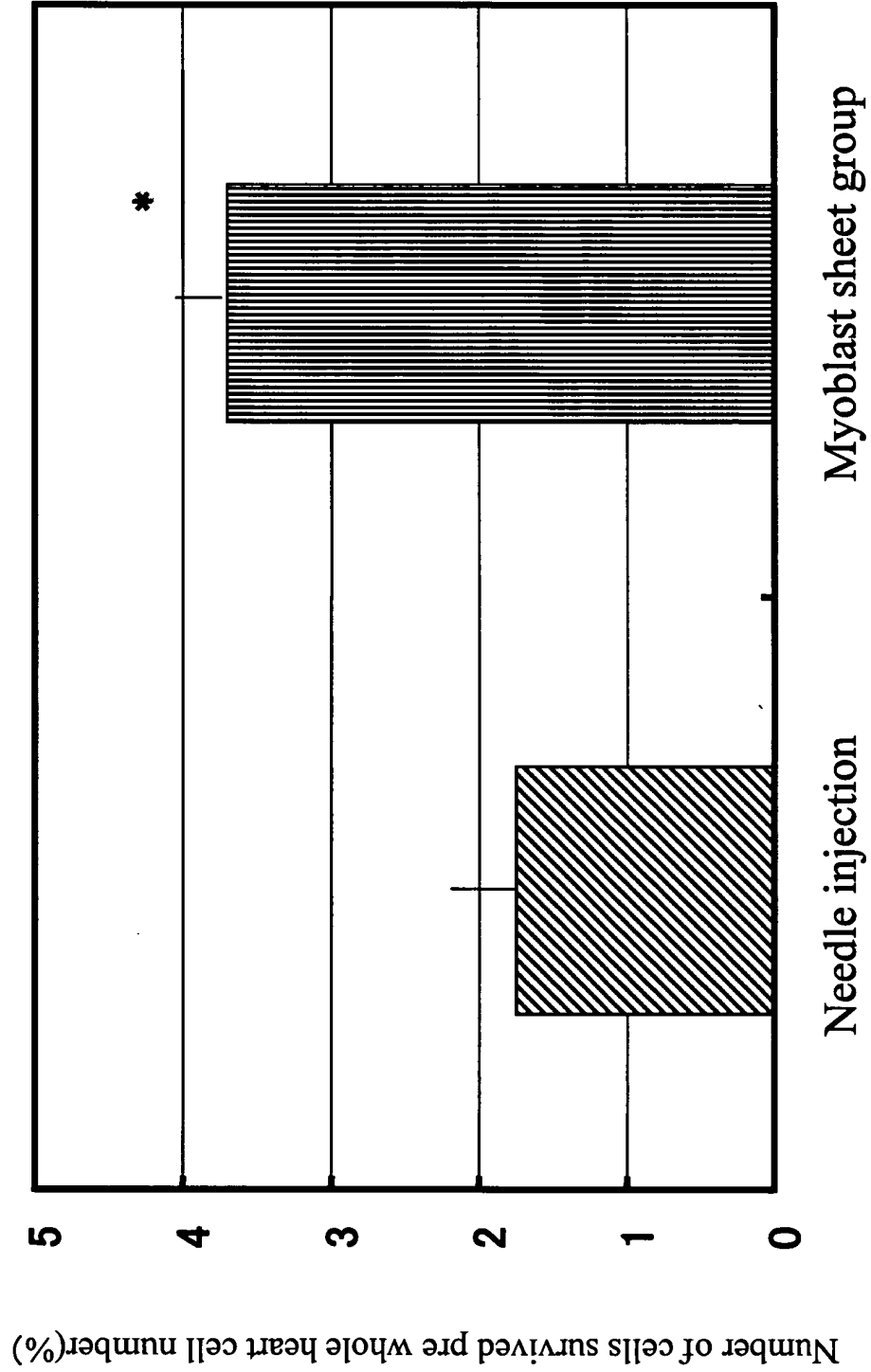


FIG.32

Electronical properties of myoblast sheet

MED system

Cardiomyocyte sheet

Myoblast sheet

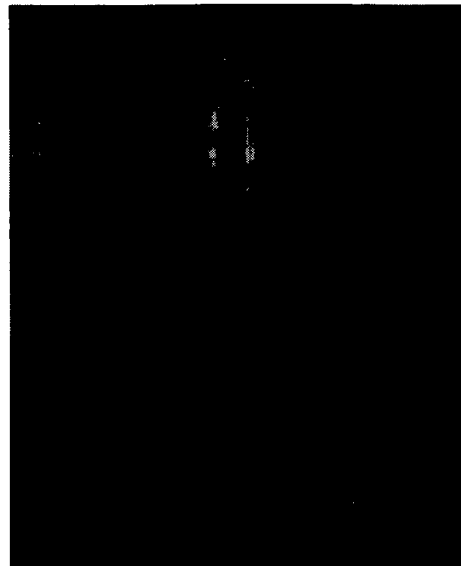
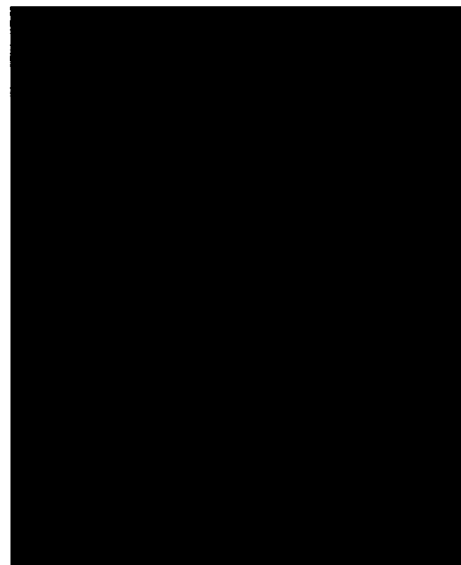
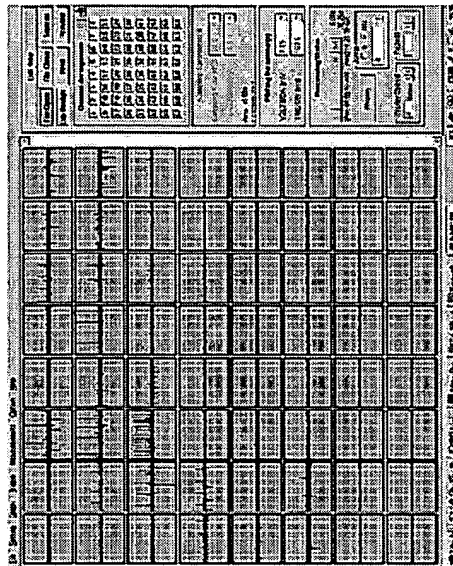
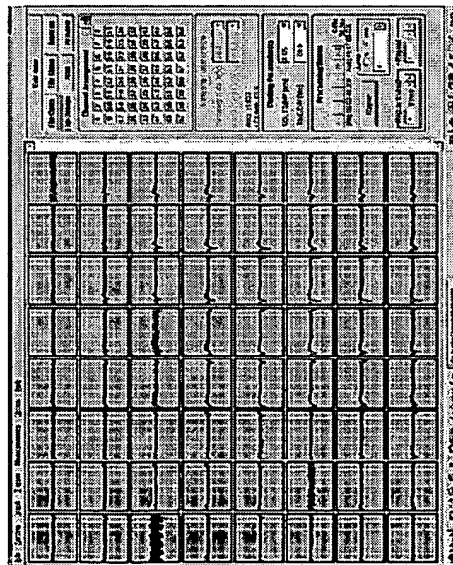
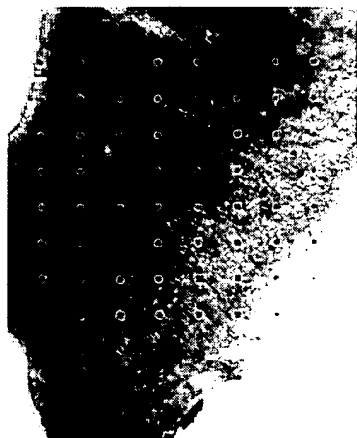
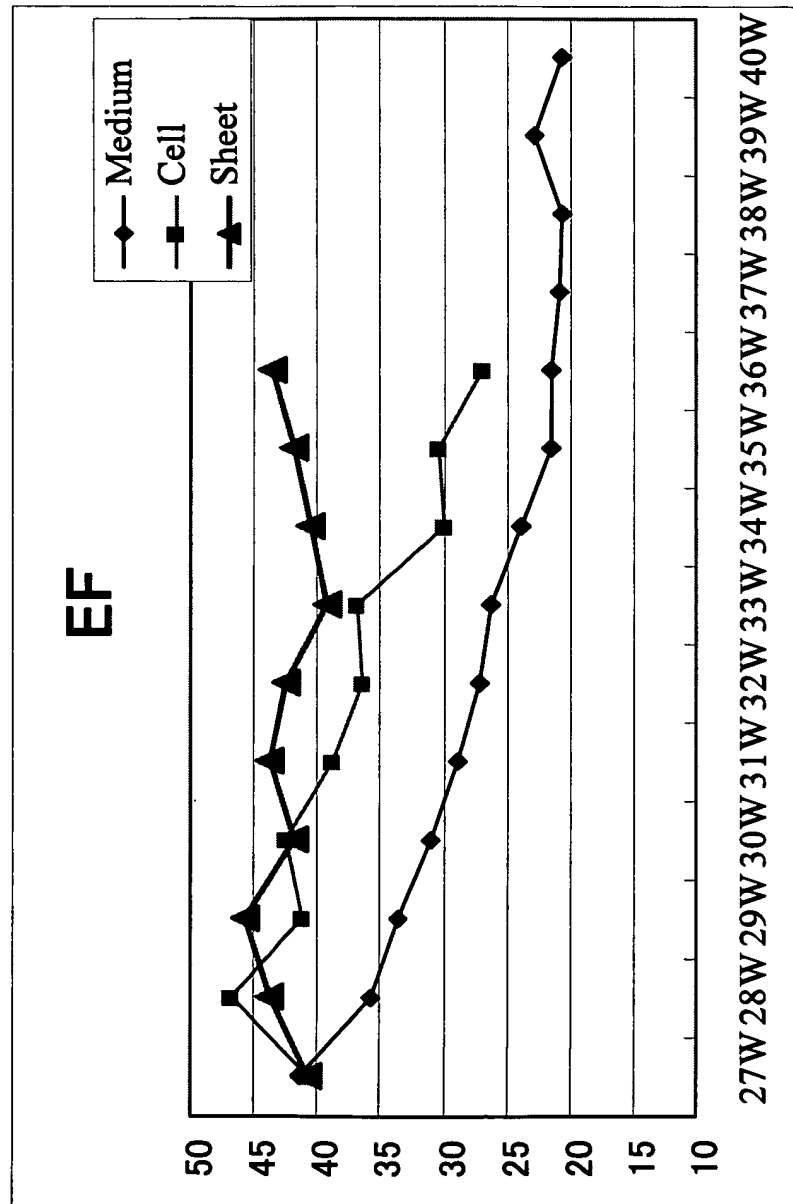
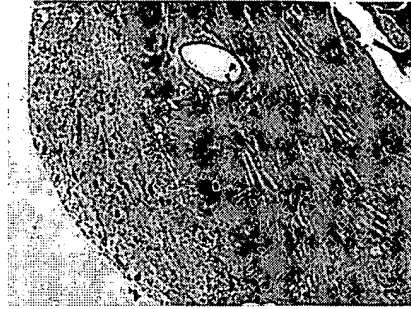


FIG.33A Myoblast sheet implantation to dilated cardiomyopathic hamster



HE staining

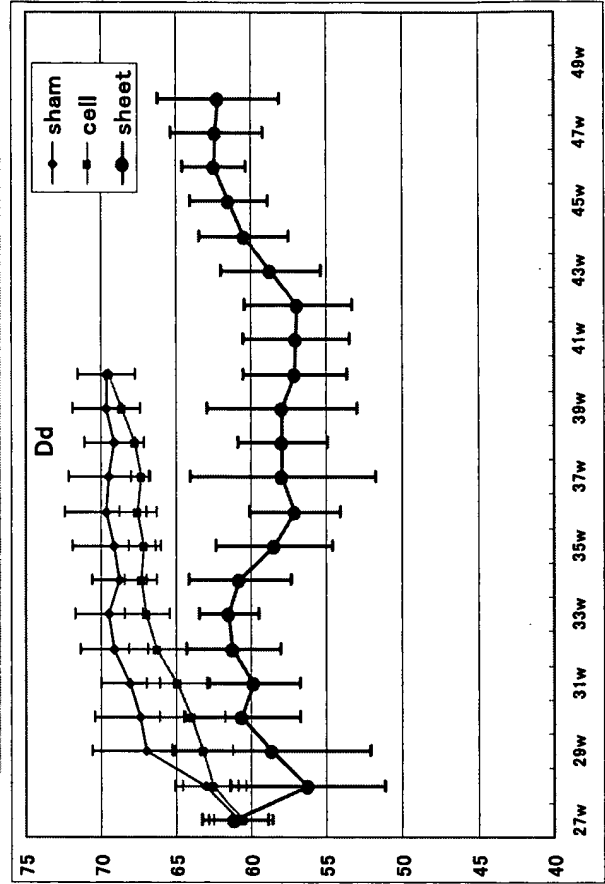


Masson's Trichrome staining



FIG.33B

Left ventricular end-diastolic diameter



Left ventricular end-systolic diameter

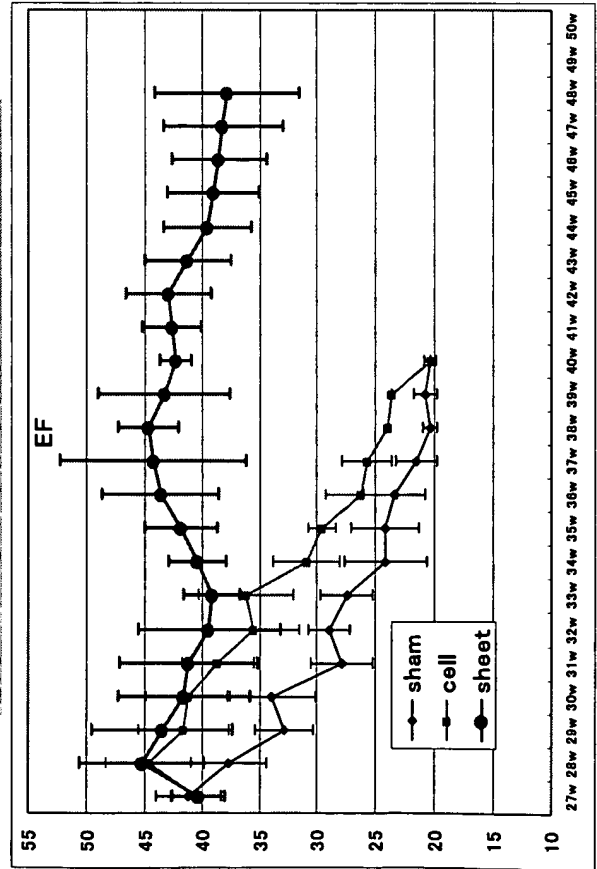
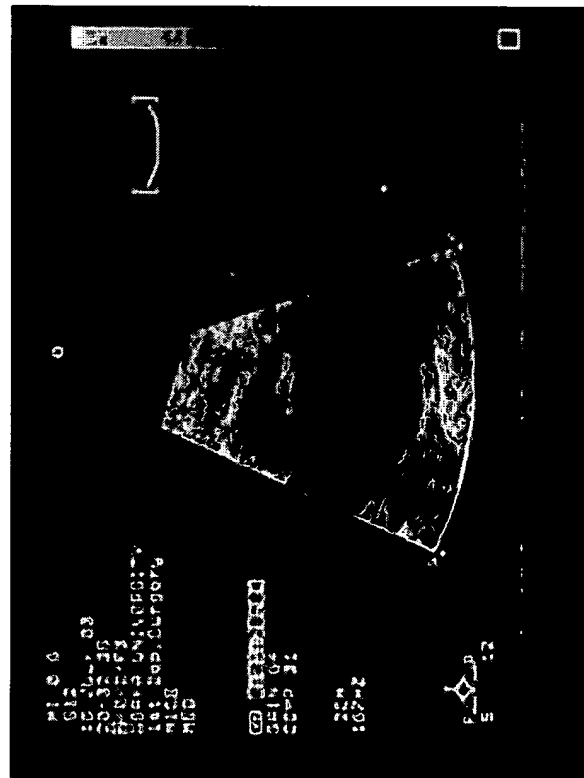


FIG. 33C

Control group



Myoblast sheet implantation group

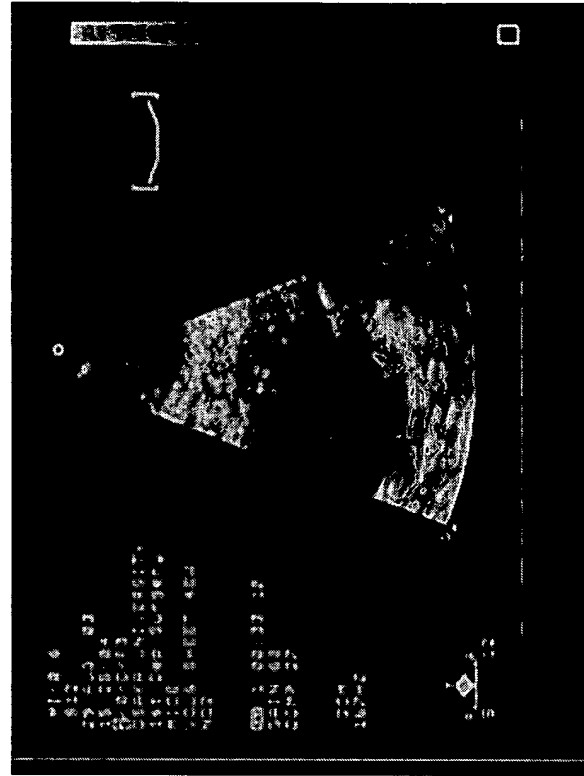
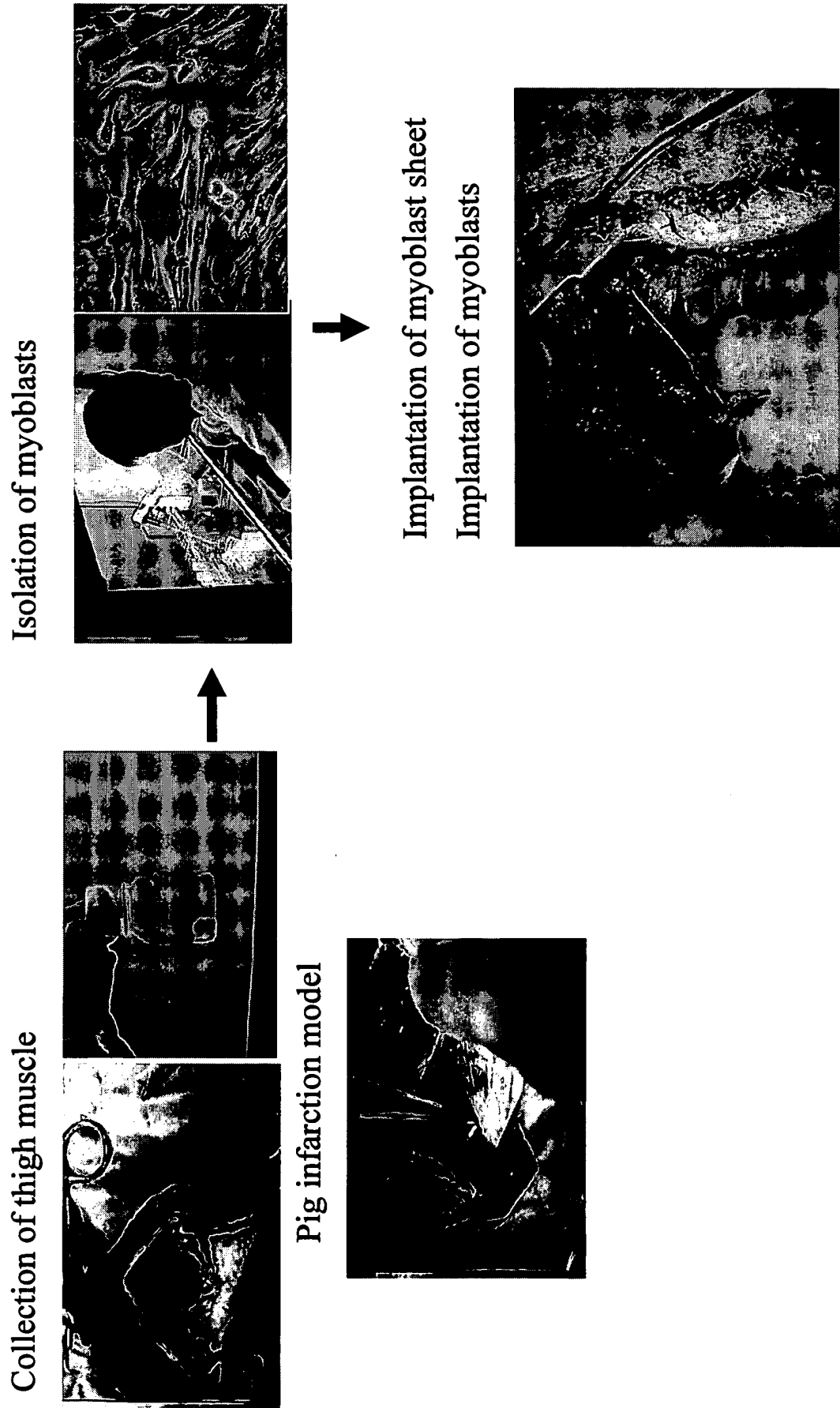


FIG.34 Myoblast sheet implantation into pig infarction model



Evaluation of cardiac function (systolic function) of
pig infarction model by CKI method

FIG.35

Before operation

After operation



Implantation
site

Evaluation of cardiac function (diastolic function) of
pig infarction model by CKI method

Before operation After operation

FIG.36

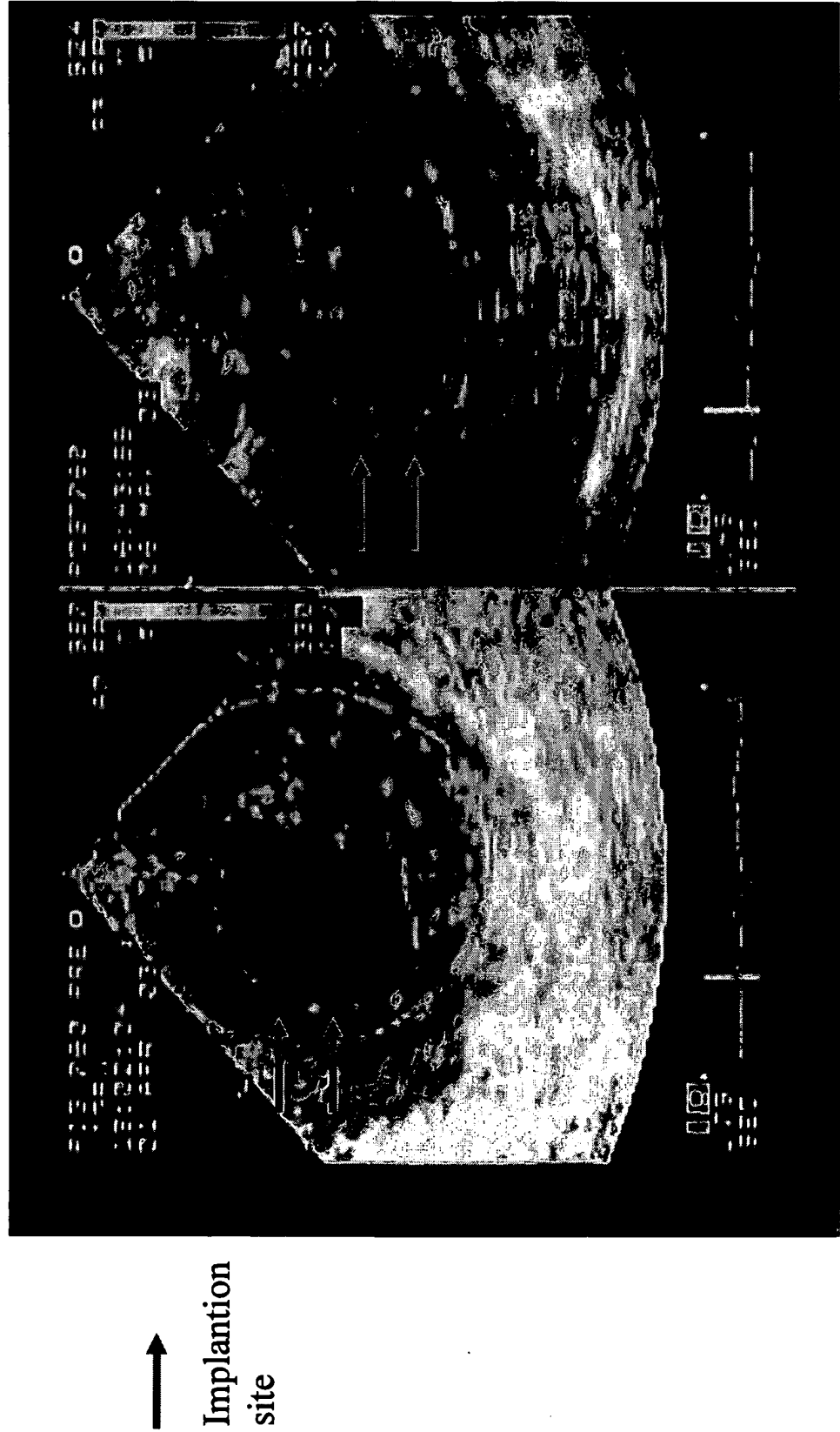


FIG.37

Without ascorbic acid



FIG.38

With ascorbic acid

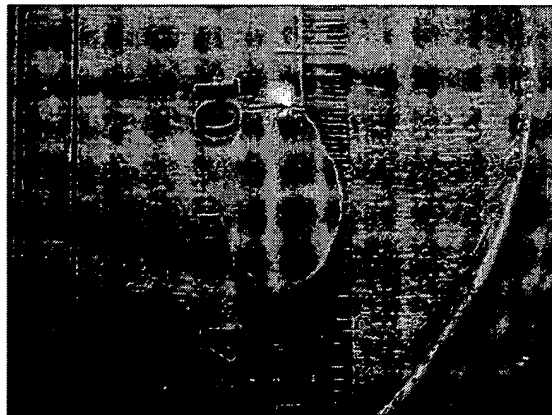


FIG.39

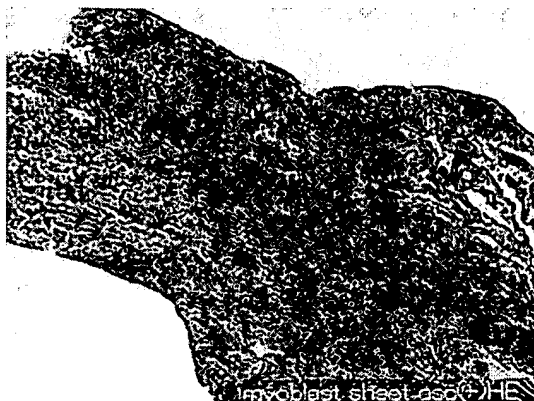


FIG.40

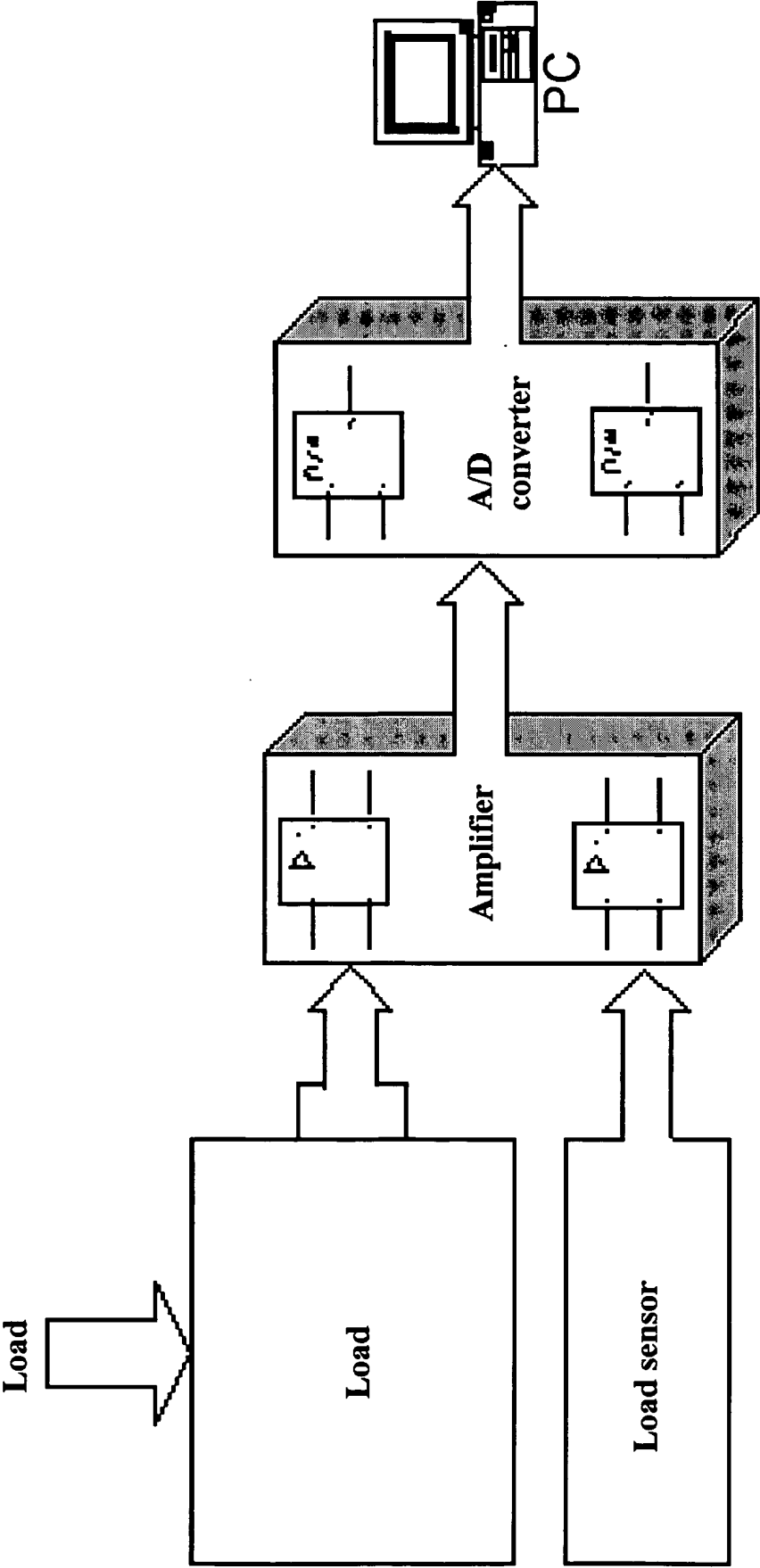
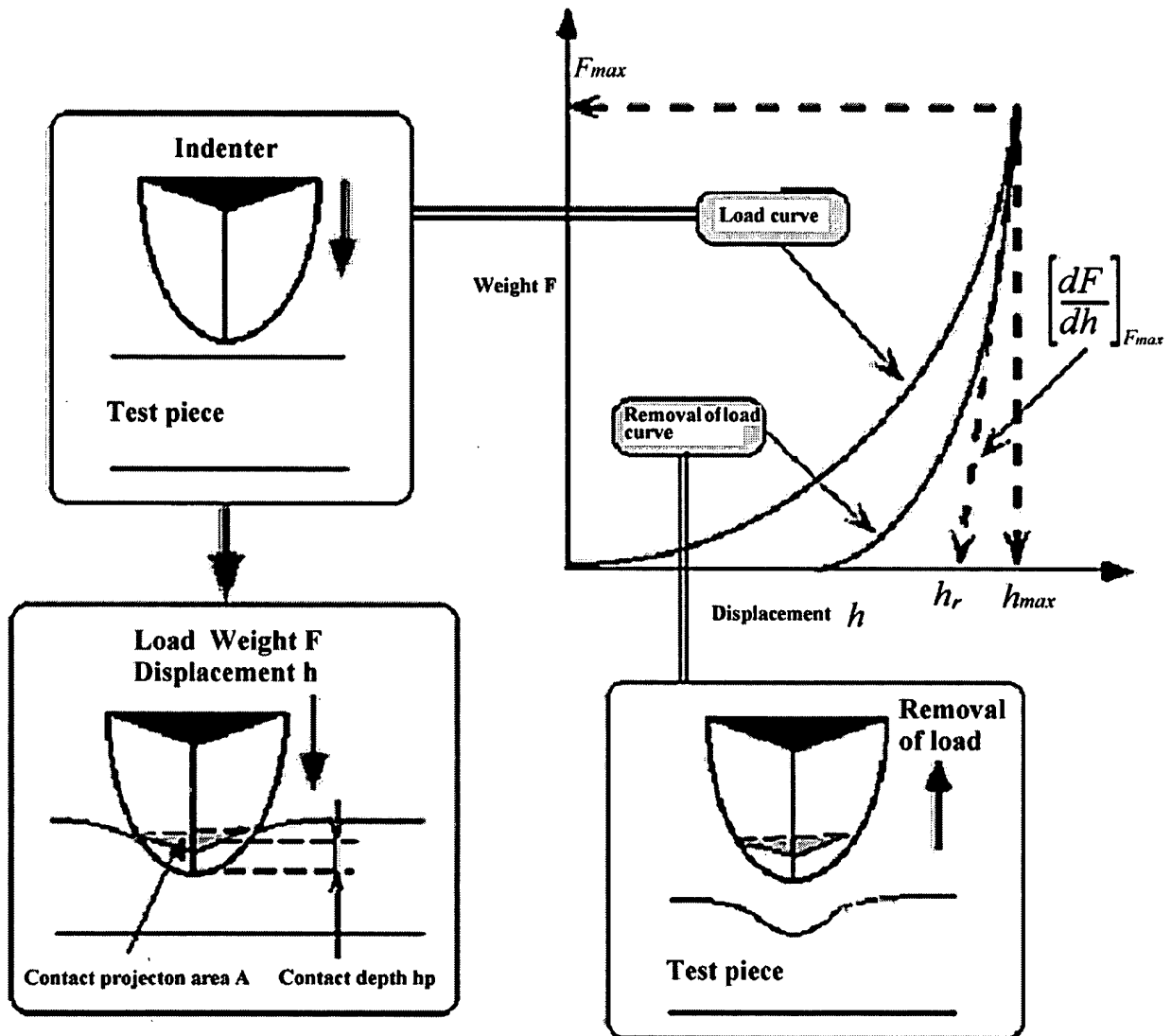


FIG.41



Rigidity $H = \frac{F}{A} = \frac{F}{k_1 h_p^2}$

Young's modulus $E = \left[\frac{dF}{dh}\right]_{F_{max}} \frac{1 - \nu^2}{2 \cdot k_2 \cdot h_{pmax}}$

Contact depth $h_p = h_r + 0.25(h_{max} - h_r)$

F : Load

A : Contact projection area

h_p : Contact depth

k_1, k_2 : Shape coefficient

F_{max} : Maximum load

h_{max} : Max. displacement

h_r : Point at which tangential line cross weight 0

dF/dh : Gradient of tangential line of the removal of load curve

ν : Poisson's ratio

FIG.42



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